APRIL 10, 1941

THE JOURNAL OF NUTRITION

VOLUME 21 NUMBER 4

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Official Organ of the American Institute of Nutrition
PUBLISHED MONTHLY BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA, PA.

Price, $5.00 per volume, Domestic; $5.50 per volume, Foreign; $6.25 per volume in Canada

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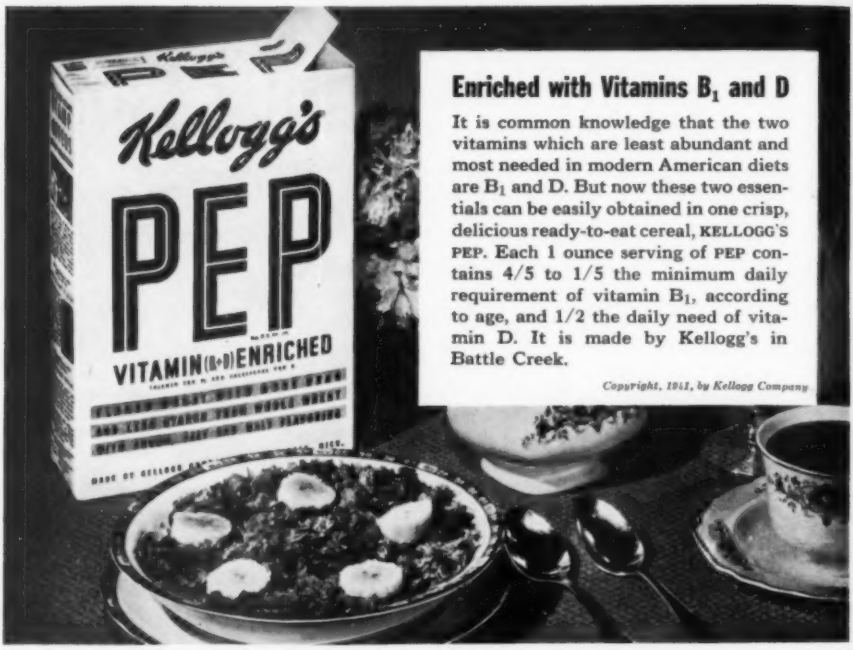
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SKIN TEMPERATURES OF THE PIG, GOAT, AND SHEEP UNDER WINTER CONDITIONS

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ONE FIGURE

(Received for publication October 12, 1940)

As the skin temperature is essentially a resultant of heat production and heat loss and as these two factors may be altered by wide ranges in environmental temperature, a study was made of the influence of relatively low (winter) environmental temperatures on the skin temperatures of animal species differing with respect to their protective coats and the insulating layers of fat under the skin. In the broad comparative studies of basal metabolism at the Laboratory for Animal Nutrition, of the University of New Hampshire, skin temperature measurements have already been made on steers (Benedict and Ritzman, '23, '27). The present report supplements the comparative picture with similar data for the goat, sheep, and pig, and these data have been compared with previous measurements of heat production of these same animal species under related conditions. As few skin temperature studies have been made on animals other than man and as our own data are not extensive, no attempt will be made to review the literature.

EXPERIMENTAL

A sow, a female goat, a ram, and a ewe, all adult, were used for the study. The ewe and the sow were pregnant. The time at which the animals last ate varied. The length of the hair of the sow and the goat at position 13 (see insert, fig. 1) was 5.1 cm. For the ewe and the ram the depth of the protective coat in its normal condition, at this point, was 8.9 and 5.4 cm., respectively.

THE JOURNAL OF NUTRITION, VOL. 21, NO. 4
APRIL, 1941

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The skin temperature was measured at the base of the hair or wool by a thermojunction, with the apparatus described by Benedict, Coropatchinsky and Finn ('28). The measurements were made on the right side at points selected to give a general picture of the average skin temperature. The results are shown graphically in figure 1. Points where few data were obtained because of the density of wool or the presence of moisture are omitted from the curves.

In this report the word "temperature" will be used to refer to skin temperature and the word "environment" to the condition of the surrounding air. All values are in degrees Centigrade. The cold environments were obtained in an animal shelter with one side open and the warmer environments in a room 3.6 by 4.6 meters, heated by a coal stove.

RESULTS

Constancy of results in two series of measurements. With the goat, under essentially similar conditions, two series of measurements (curves 3 and 4) were made an hour apart, at 18° and 21°. The temperatures on the same spot differed by 1.3° and 1.8° at two points on the head, but all others on any one spot agreed within 1°C. The average temperatures were the same. With the ewe two series at 14° (curves 7 and 8), made 20 minutes apart, showed a greater maximum variation at any one point, possibly due to local exposure in making the measurements, but the averages in these two series were only 0.3° apart. Usually measurements under like conditions could be duplicated within 1°C.

Adaptation to a given environment. Measurements were made on the goat immediately after it had walked about 18 meters from an environment of —12° to a room at 19° (curve 2) and again 1 and 2 hours after being in the warm room (curves 3 and 4). The first series agreed well with the two subsequent series, although averaging 0.3° higher (possibly because of the physical exertion of walking). Seventeen hours later, during which the environment had gradually fallen 5° lower, the temperatures (curve 5) averaged 2.3° lower. Hence the goat adjusted itself rapidly to changes in environment.

The pig, after observations in the barn at —12°, was measured again immediately after walking to the warm room at 19° and also 20 hours later (curves 11, 12, and 13). During these 20 hours the environment descended to 13°C. Although the later measurements were made at an environment 5° below the initial level in the warm room, the temperatures averaged 3.4° warmer than the initial temperatures. Hence with the pig the adjustment to changes in environment was not immediate, as it was with the goat.

Effect of differences in environment on the temperature of the same animal. Measurements on the ewe at 14° and —3° (curves 7, 8, and 6) showed an average difference of about 5° in the temperature of the trunk, but even at the freezing environment (—3°) the temperature on this portion of the body was still well above 30°C. The differences on the head were almost equivalent to the difference in environment. The average trunk temperature of the goat was 1.9° lower at 16° (curve 5) than at 21° (curve 4) but at —12° (curve 1) was only 4.5° below that at 16°C. The temperatures on the head at —12° were influenced to a greater degree by the environment than were those on the trunk. The temperature of the pig's trunk averaged 10° lower at —3° (curve 10) than at 14° (curve 13), and the additional decrease of 9° in environment, i.e., to —12° (curve 11), resulted in a further decrease in trunk temperature of 4.5°C. The temperature of the pig's head was more uniform than that of the trunk, and at the —12° environment the head was generally warmer than the trunk.

Comparison of average temperatures of the different animals at various environments. Under essentially similar environmental conditions (14° to 16°) the temperatures of the sheep were higher on all portions of the body than those of the goat and the temperatures of the goat were higher than those of the pig. The average trunk temperatures at the different environments were as follows:

Environment	—12°	—3°	+3°	14°	16°	18°	21°
Goat	29.4°	—	—	—	33.9°	35.6°	35.8°
Sheep	—	31.7°	33.2°	36.3°	—	—	—
Pig	15.4°	19.9°	—	29.8°	—	—	—

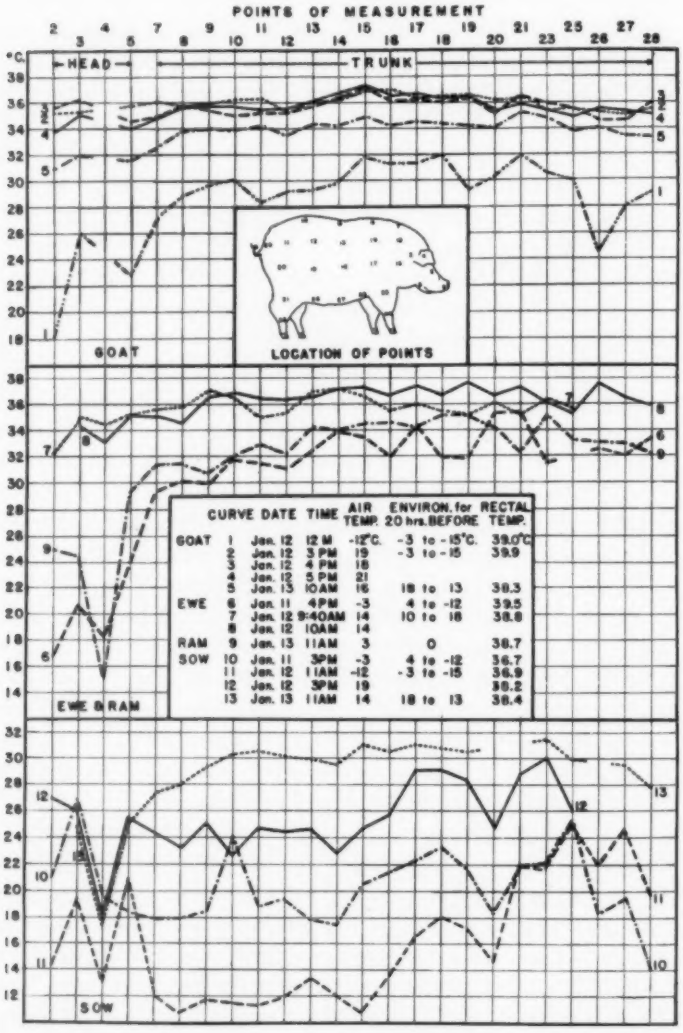
POINTS OF MEASUREMENT
HEAD
TRUNK
°C.
GOAT
LOCATION OF POINTS
EWE & RAM
SOW
CURVE DATE TIME AIR TEMP. ENVIRON. for 20 hrs. BEFORE RECTAL TEMP.
GOAT 1 Jan. 12 12 M -12°C. -3 to -15°C. 39.0°C
2 Jan. 12 3 PM 19 -3 to -15 38.9
3 Jan. 12 4 PM 18
4 Jan. 12 5 PM 21
5 Jan. 13 10AM 16 18 to 13 38.3
EWE 6 Jan. 11 4PM -3 4 to -12 39.5
7 Jan. 12 9:40AM 14 10 to 18 38.8
8 Jan. 12 10AM 14
RAM 9 Jan. 13 11AM 3 0 38.7
SOW 10 Jan. 11 3PM -3 4 to -12 36.7
11 Jan. 12 11AM -12 -3 to -15 36.9
12 Jan. 12 3PM 19 38.2
13 Jan. 13 11AM 14 18 to 13 38.4

Fig. 1 Skin temperatures of farm animals on different parts of the body, as influenced by different environmental temperatures. The upper insert shows the positions of measurements for the sow; corresponding positions were used for the ram, ewe, and goat. The lower insert gives the conditions for the individual curves, air temperature indicating that during the measurement.

Clearly the sheep and the goat can maintain their temperatures relatively uniform over wide ranges in environment, as compared with the variability found with the pig.

Comparison of metabolism and skin temperature at different environments. Metabolism measurements made previously on similar adult animal species (Ritzman et al., '30, '31, '36) under contrasting environmental conditions, when quiet and fasting, show that the average heat production, expressed as calories per kilogram of body weight per 24 hours, is as follows:

SHEEP		GOAT [1]		PIG [1]	
°C.	Cals.	°C.	Cals.	°C.	Cals.
0 to 3	29.8	8	32.3	12	19.0
6 to 9	29.5	12 to 16	28.5	18 to 24	19.0
21 to 27	30.2	20 and over	23.9		

[1] Unpublished data obtained at the Laboratory for Animal Nutrition, University of New Hampshire.

The heat production of sheep was not affected by the environment, at least down to 0°. This, coupled with the finding that its temperature was essentially uniform over a wide range in environment, emphasizes the excellent protection afforded by the fleece. When the fleece was removed, the heat production increased. The metabolism of the goat increased immediately when the environment dropped below 20°, as was the case also with sheared sheep. Hence the relative uniformity in the goat's temperature even in a freezing environment may be ascribed both to its increased metabolism and to the protection afforded by its hair. The heat production of the pig remained the same at 12° as at 20° or over. Its skin temperature fluctuated with environmental changes, due to its poor hair coat, but it possessed a protection against cold in the form of large fat deposits in and under the skin. With this insulation its heat production remained uniform between 12° and 24°. These observations show that changes in skin temperature cannot be used as an indication of metabolic changes and that differences in the external protective coat and the fat insulation in and beneath the skin may account for wide variations in skin temperature not correlated with heat production.

SUMMARY

Skin temperature measurements were made on two sheep, a pig, and a goat at environmental temperatures between —12° and +21°C. Duplicate measurements on the same spot on the body usually agreed within 1°C. Equilibrium in skin temperature after change to a different environmental temperature was quickly established with the goat but more slowly with the sow. The ewe, the ram, and the goat maintained their skin temperatures near 30°, even when the environmental temperatures were at the freezing point or below. The skin temperature of the sheep was warmer than that of the goat or the pig under all conditions, the goat's temperature being slightly lower and the pig's appreciably lower. The average skin temperatures on the trunk were as follows:

ENVIRONMENT	GOAT	SHEEP	PIG
14° to 16°	33.9°	36.3°	29.8°
+ 3°	—	33.2°	—
— 3°	—	31.7°	19.9°
—12°	29.4°	—	15.4°

The protective coats of the sheep and the goat enabled them to maintain their skin temperatures within relatively narrow ranges despite large differences in environment, but the poorer external protection of the pig did not enable such uniformity.

Comparison is made between these skin temperature measurements and metabolism measurements previously made on similar animal species over a wide range in environmental temperatures.

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PEROSIS DUE TO A VITAMIN DEFICIENCY[1]

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NINE FIGURES

(Received for publication October 29, 1940)

Hogan, Guerrant and Kempster ('25) observed that chicks reared on simplified rations frequently developed the deformity of the legs later known as hock disease, slipped tendon, or perosis. Hogan and Shrewsbury ('30) observed that these symptoms are prevented by wheat or by wheat middlings and attempted to determine whether the protective action is due to mineral constituents. Accordingly wheat was ignited and the ash of 100 gm. of wheat was incorporated in 100 gm. of the experimental ration, but this addition was entirely ineffective. It was concluded that the abnormality was due to the deficiency of an organic constituent. In the meantime Wilgus, Norris and Heuser ('37 a) reported that perosis is due to a deficiency of manganese, an observation that has been amply confirmed.

At first it seemed difficult to reconcile these conflicting claims. Substances which in our experience prevented perosis lost their effectiveness when ignited. On the other hand the Cornell group almost entirely eliminated perosis merely by including a minute amount of manganese in the ration. In order to explain this discrepancy it was assumed that in addition to manganese an organic substance is required to prevent perosis. Our rations were deficient in the organic factor,

[1] Contribution from the Missouri Agricultural Experiment Station, Journal Series no. 699.

those of Wilgus, Norris, and Heuser were deficient in manganese. Hogan, Richardson, and Patrick ('40) were able eventually to obtain evidence that sustains our hypothesis. This paper is to present the evidence in more detail.

EXPERIMENTAL

Experimental procedure. The baby chicks were approximately 1 day old when delivered from the hatchery, and they were supplied with the experimental rations immediately. The experimental procedure has been described by Hogan and Boucher ('33).

Preparation of vitamin carriers. We had never succeeded in formulating a synthetic diet that is adequate for the chick unless liver extracts were included, and the chicks which received these rations seldom, or never, developed perosis. In order to detect the presence of an organic perosis-preventing substance it would be necessary to separate the liver extract into two fractions, one of which contains this hypothetical factor. The second fraction would not prevent perosis but it must contain the other vitamins that are necessary to maintain the chick in a reasonably satisfactory nutritional state.

The procedure as finally adopted is fairly simple:

1. Fresh beef liver is ground in a meat chopper, and dried in a current of air at a temperature of 55 to 60°C. The dried product is reground to a fine state of subdivision.

2. The dry liver powder is extracted at a temperature of 70°C. with 95% ethyl alcohol. The extracts are filtered hot and allowed to cool, when a considerable amount of lipoidal material separates and is discarded. The alcoholic solution is concentrated in vacuo until it begins to foam badly, then poured into cold water when a fatty fraction should rise to the top. Whether it does or not the mixture is warmed to 70°C. and then the fatty layer separates sharply and is skimmed off and discarded. The aqueous phase is filtered through several layers of cheese cloth and concentrated in vacuo to a dry matter content of about 50%. This is preparation 4303.

3. The liver powder residue from step 2 is extracted exhaustively with boiling 95% ethyl alcohol. This extract is not a highly active source of the perosis-preventing factor, and has not been extensively used.

TABLE 1

Composition of rations

CONSTITUENT	RATION NO.				
	4121	4507	4928	4970	4976
Casein	35	35	25	35	35
Corn starch	25	21	35	24	22
Cane molasses				5	5
Lard	7	7	15	15	15
Soybean oil	8	8			
Cellulose	3	3	3	3	3
Salts (3516)[1]	4	4	4	4	4
Cod liver oil	2	2	2		
A-D mixture[2]				2	2
Liver residue (4081)	10	10	10	10	10
Alcohol extract (4303)		4			
Water extract (4080)	6	6	6	2	4
$MnSO_4$, $4H_2O$, gm.	0.1	0.1	0.1	0.1	0.1
Thiamine[3], μg.	200	200	200	200	
Riboflavin, μg.	400	400	400	400	
Vitamine B_6, μg.		300	500		
alpha-tocopherol, mg.			8	8	8
2-methyl-1,4-naphthoquinone, mg.			1	1	1

[1] Salts 3516, Hubbell, Mendel and Wakeman ('37).

[2] A concentrate of vitamins A and D dissolved in lard. According to the manufacturers' assay 1 gm. of the mixture contains 3000 units of vitamin A and 425 units of vitamin D.

[3] The vitamins were generously supplied by Merck and Co., Rahway, New Jersey.

4. The liver powder residue from step 3 is extracted with tap water previously heated to boiling. The extracts are combined, filtered, and concentrated in vacuo to a dry matter content of about 50%. This is preparation 4080.

This preparation should contain little or none of the perosis-preventing factor, but in order to attain a satisfactory degree of separation it is necessary that the preceding extraction with alcohol be complete.

5. The residue remaining after extraction with hot water is preparation 4081.

Typical experimental rations are described in table 1.

The alcohol extract of liver prevents perosis. Since the extent of the deformity is variable the degree was estimated by the method of Wilgus, Norris and Heuser ('37 b). According to this system the maximum degree is 100.

The response of the chicks to the two types of liver extracts is shown in table 2. Ration 4121 contains the water extract of

TABLE 2

Response of chicks to various rations, at close of a 6-week experimental period

GROUP AND RATION	BREED	FEMALES		MALES		PEROSIS		MORTALITY
		No.	Weight	No.	Weight	Degree	Incidence	
			gm.		*gm.*	%	%	%
I 4121	W.L.[1]	15	240	13	198	67	100	37
	N.H.R.	4	268	1	258	51	100	0
	W.R.	12	208	11	164	57	100	30
II 4121 + 300 μg. vitamin B_6	W.L.	1	290	2	412	46	100	
	N.H.R.	2	572	1	654	52	100	
	W.R.	6	333	3	406	66	100	
III 4121 + 500 μg. vitamin B_6	W.L.	3	384	8	405	33	100	
	W.R.	5	470	6	480	52	100	
IV 4507	W.L.	7	410	7	459	0	0	
	N.H.R.	2	553	3	549	0	0	
	W.R.	5	512	6	640	0	0	
V 4121, Mn omitted	W.R.	3	183	3	218	48	100	
	N.H.R.	2	108	2	215	97	100	25
VI 4121, Mn omitted + 500 μg. vitamin B_6	W.R.	3	250	3	358	92	100	
VII 4507, Mn omitted	W.R.	2	549	2	583	0	0	
VIII 4121, Mn at minimum	W.R.	6	150	6	126	47	100	41
	N.H.R.	2	287	2	289	81	100	0
IX 4121, Mn at minimum + 500 μg. vitamin B_6	W.L.	4	295	6	319	80	100	
	W.R.	3	285	6	320	80	100	
X 4507, Mn at minimum	W.L.	5	397	5	450	14	70	
	N.H.R.	2	540	2	625	48	100	
	W.R.	6	461	6	609	8	8	

[1] W.L.=White Leghorn. N.H.R.=New Hampshire Reds. W.R.=White Rocks.

liver but not the alcohol extract. Symptoms of perosis appeared at the end of the second week and by the end of the fourth week all chicks which survived had well developed symptoms. The chicks grew slowly and the mortality was high. Since the symptoms suggested that the mortalities were due to a deficiency of vitamin B_6, this vitamin was added to the basal ration (groups II and III). Addition of 300 or 500 μg. largely eliminated the mortalities, and accelerated the growth rate, but it did not reduce either the incidence or degree of perosis. Apparently the chick requires between 300 and 500 μg. of vitamin B_6 for the optimum rate of growth.

The chicks just described received the water extract of liver and developed perosis. The next group received ration 4507 which contains both the water and alcohol extracts, and every chick was normal. An example of perosis is shown in figure 9.

Effect of a manganese deficiency. According to Gallup and Norris ('39) the amount of manganese required for normal bone development is approximately fifty parts per million. A study was made, therefore, to determine the effectiveness of the alcoholic extract when the ration is deficient in manganese. The first ration with a low manganese content (group V) contains twenty parts per million. This ration contains the water extract of liver but not the alcohol extract. Symptoms of perosis appeared before the end of the second week and by the end of the third week the condition was well developed. The chicks grew slowly and the mortality was high. Again there was reason to believe that the mortalities were due to a deficiency of vitamin B_6, and therefore 500 μg. per 100 gm. were added to the diet (group VI). The addition largely eliminated the mortalities and accelerated the growth rate, but it did not reduce either the incidence or degree of perosis.

Group VII received a ration which contains both the water and alcohol extracts. It will be observed that the incidence of perosis is zero, and of course the degree is zero. The only breed of chicks tried was the White Rock. In this case perosis was completely prevented by supplying the chicks with the

alcoholic extract of liver though the manganese level was approximately 30 p.p.m.

An attempt was now made, with little success, to prepare a ration (group VIII) with a minimum manganese content by omitting manganese from the salt mixture. The new mixture was the same as mixture 3516 in all other respects. However, as shown in table 3, the amount of manganese in mixture 3516 was so low that omitting it entirely had no detectable effect on the analysis. The ration of group VIII contained the water extract of liver but not the alcohol extract. The chicks grew slowly, therefore vitamin B_6 was added at a level of 500 μg. per 100 gm. (group IX). This addition brought about vast improvement in the condition of the chicks but the incidence and degree of perosis was about the same as when the vitamin was omitted.

Group X received both the water and alcohol extract but again the manganese content was reduced to a minimum by omitting all inorganic compounds of this element from the ration. It will be observed that the incidence of perosis was 70% for the White Leghorns, 100% for the New Hampshire Reds and only 8% in the White Rocks. Apparently breeds that mature early are more subject to perosis. It also seems evident that when the ration is deficient in manganese, perosis cannot be prevented by supplying the alcoholic extract, though this constituent does reduce the degree of severity. Both manganese and the extract are required to entirely eliminate this abnormality.

The data have been scrutinized to see whether susceptibility to perosis is affected by sex, but this factor is apparently of little or no significance. It is our experience that when the ration is deficient in the organic perosis-preventing substance all breeds are equally susceptible. As was previously mentioned though, there is a differential breed susceptibility when manganese is deficient.

Substitution of other minerals for manganese. According to Wilgus, Norris and Heuser ('37 a) the addition of iron, zinc, and aluminum to perosis-producing rations is somewhat

effective in reducing the severity of the symptoms. These elements, each at a level of 0.0025% of the ration, were added to ration 4121. These additions increased the amounts of iron and zinc in the complete ration only slightly, but increased the total amount of aluminum by almost 70%. This modification did not affect either the incidence or the degree of perosis. Lyons, Insko and Martin ('38) also obtained negative results with these elements.

In the past perosis has been ascribed to the provision of an insufficient quantity of manganese, or to the provision of excessive amounts of calcium and phosphorus, or to unsuitable proportions of the mineral elements. For that reason we are reporting in table 3 a partial mineral analysis of the rations used to demonstrate that perosis was due to deficiency of an organic factor. It will be observed that the rations which prevent, or do not prevent, perosis may contain essentially the same percentages of the inorganic constituents. It is especially significant that a ration such as no. 4121 may contain five times as much manganese as the chick requires, and still permit every one that consumes it to develop perosis.

TABLE 3

Mineral content of rations [1]

GROUP AND RATION	CALCIUM	PHOSPHORUS	MANGANESE	IRON [2]	ZINC [2]	ALUMINUM [2]
	%	%	%	%	%	%
I 4121	0.86	0.99	0.027	0.037	0.028	0.0036
IV 4507	0.86	1.02	0.028	0.048	0.032	0.0017
V 4121, Mn omitted	0.86	0.99	0.002	0.037	0.028	0.0036
VII 4507, Mn omitted	0.86	1.02	0.003	0.048	0.032	0.0017
VIII 4121, Mn at minimum	0.86	0.99	0.002	0.037	0.028	0.0036
X 4507, Mn at minimum	0.86	1.02	0.003	0.048	0.032	0.0017

[1] Determined by calculation from the analysis of the individual constituents.
[2] Determined spectrographically by Dr. V. R. Ells.

Measurements of bones. Caskey, Gallup and Norris ('39) have shown that chicks with perosis have leg and wing bones that are abnormally short and thick. Since the dimensions provide an objective criterion of perosis a considerable number of measurements were taken. The chicks were White Rocks with the exception of six which were New Hampshire Reds. The chicks were sacrificed and the humerus, tibia, and metatarsus were immediately dissected out with the cartilage caps in place. At first the bones of both the left and right sides were measured, but since the measurements of corresponding bones of the same chick were practically identical the later practice was to take measurements on only one side. The length of the humerus was obtained by measuring the distance between the trochlea and the oval articular head at the proximal end. The length of the tibia was obtained by measuring the distance between the distal and proximal extremity and the length of the metatarsus was obtained by measuring the distance between the trochlea for inner digit and the proximal extremity. The diameter was measured in the region of the shaft that appeared to be the most narrow. The shaft was placed in the calipers and turned, the highest reading being recorded.

Most of the chicks with perosis were smaller than the controls and after eliminating those in which the disparity in size was too pronounced only eleven pairs remained that had approximately the same weight. The weights of the twenty-two chicks ranged from 180 to 654 gm. The average weights and measurements of the eleven pairs on the two diets are shown in table 4. The differences between the two sets of

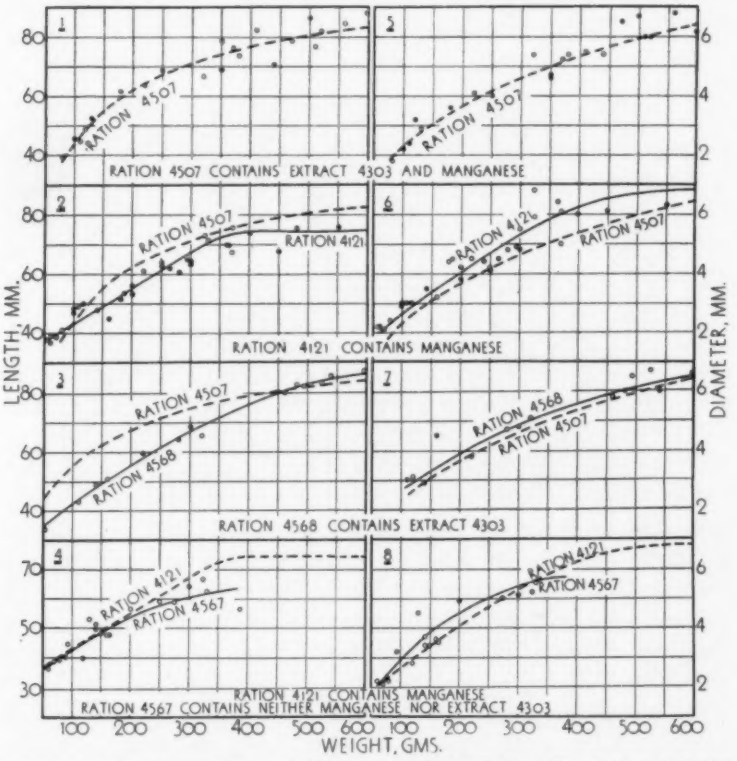
TABLE 4

Weights and bone measurements of chicks on the control and on the perosis-producing ration

RATION NO.	WEIGHT PER CHICK	LENGTH			DIAMETER		
		Humerus	Tibia	Metatarsus	Humerus	Tibia	Metatarsus
	gm.	mm.	mm.	mm.	mm.	mm.	mm.
4121	382.7	48.6	69.2	50.1	5.6	6.0	6.8
4507	383.6	54.8	75.3	54.5	5.1	5.0	5.9

measurements are entirely consistent. In every case the bones of chicks which did not receive the alcohol extract were shorter and thicker than those from chicks which did receive it.

The measurements of the tibia from chicks on four different rations are plotted against weight in figures 1 to 8. In some cases the variability is so great that the exact position of the smoothed curves is of no great significance, but they have been drawn to facilitate comparisons. The chicks that received



Figs. 1 to 8 Measurements of tibia, from White Rocks. The data points for chicks on ration 4507 appear only in figures 1 and 5, for chicks on ration 4121 they appear only in figures 2 and 6.

It will be noted that when the alcohol extract is omitted the abnormality is most marked. When only manganese is deficient the degree of perosis is intermediate. Ration 4567 was supplied to group VIII, ration 4568 to group X, of table 2.

ration 4507, which contained the alcohol extract and liberal amounts of manganese, were normal, and their bones were taken as a standard.

Ration 4121 contained a liberal amount of manganese, but did not contain the alcohol extract. The bones of chicks which received this ration were shorter and thicker than normal.

Ration 4568 contained the alcohol extract but was deficient in manganese. At the lower weights (and ages) the bones were abnormally short, and to a lesser degree they were abnormally thick. At the higher weights (6 weeks of age) the bones were normal in length and apparently they were also normal in diameter.

Under the experimental conditions described it seems that a deficiency of the alcohol extract alone, was more decisive in producing abnormal bones than was a deficiency of manganese, but it may be that the deficiency in the mineral was not sufficiently acute. Gallup and Norris ('39) report that perosis in White Leghorns was completely prevented on rations that contained thirty parts per million of manganese.

Ration 4567 did not contain the alcohol extract and was deficient in manganese. As would be expected the bones were abnormally short and abnormally thick. A comparison of these chicks with those which received ration 4121 would be of interest since neither ration contained the alcohol extract, but the comparison is difficult because the deficiency of manganese depressed the rate of growth. Such data as are available indicate that the bones of chicks which received ration 4567 were distinctly the shorter at the higher weights; the diameter was apparently greater at the lower weights, and less at higher weights. The differences are too uncertain to be of much significance.

On gross inspection the symptoms of perosis are highly variable. In the same group of chicks it may be observed that: (1) The tendon of Achilles is out of position, and the tarsometatarsal bone is rotated in such a way as to point the toes to one side; (2) the tibial-tarsometatarsal joint is greatly enlarged; (3) the tarsometatarsal bone is markedly short and

thick; (4) because of curvature of the tarsometatarsal bone the chicks are bow-legged. Curvature of the tibia is less pronounced; (5) the long bones are all shorter and thicker than is normal, but the deformity would pass unnoticed on casual examination. In our experience the fifth set of symptoms is by far the most common. Each of the first four may be exhibited by a small per cent of the total number, though an occasional chick will develop them all. This wide variety of symptoms suggests the possibility that the organic factor concerned is multiple in nature.

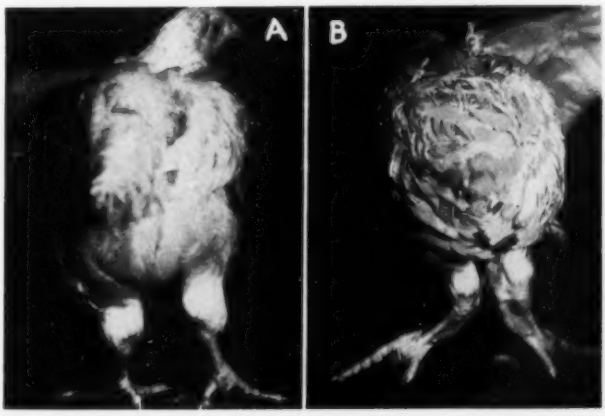


Fig. 9 A, normal chick. B, example of perosis. The joints and bones are enlarged, and the tendon of Achilles on the left leg is out of position.

Choline as a perosis-preventing agent. It has been shown by Griffith and Wade ('39) and by Abbott and De Masters ('40) that choline has unique physiological properties and it was included in some of the perosis-preventing rations. In the meantime Jukes ('40) had shown that choline at a level of 0.15 to 0.30% prevents perosis in turkeys.

Several groups of White Leghorn chicks received perosis-producing rations, and others received the same ration but with 0.1% of choline added. At the close of the experimental

TABLE 5

The effect of choline on bone development

GROUP AND RATION	CHICK NO.	WEIGHT	LENGTH			DIAMETER		
			Humerus	Tibia	Metatarsus	Humerus	Tibia	Metatarsus
			mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
4976	414M	460	52.0	77.7	55.1	5.4	5.5	5.8
A 4976 + choline	418M	470	53.4	83.2	59.2	4.9	4.6	5.4
Normal[1]	347M	485	56.5	80.5	58.2	5.0	4.9	5.7
4976	414M	460	52.0	77.7	55.1	5.4	5.5	5.8
B 4976 + choline	420M	495	55.3	81.0	59.3	5.6	5.8	6.4
Normal	347M	485	56.5	80.5	58.2	5.0	4.9	5.7
4976	416F	362	47.7	68.0	48.9	5.0	5.6	5.8
C 4976 + choline	421F	370	51.6	75.0	53.5	4.8	4.1	5.2
Normal	346F	368	49.9	68.5	50.2	4.7	4.3	5.4
4976	417F	335	43.8	65.2	45.5	6.1	5.4	6.2
D 4976 + choline	422F	300	49.6	70.3	50.1	4.6	4.2	4.9
Normal	436F	320	49.4	72.6	50.1	4.1	3.9	4.6
4976	414M	460	52.0	77.7	55.1	5.4	5.5	5.8
E 4970 + choline	363M	465	54.4	78.6	58.1	4.9	5.5	5.9
Normal	347M	485	56.5	80.5	58.2	5.0	4.9	5.7
4976	414M	460	52.0	77.7	55.1	5.4	5.5	5.8
F 4970 + choline	366M	500	52.8	76.1	56.6	5.2	5.5	6.1
Normal	374M	505	58.7	88.7	65.5	5.1	5.0	5.5
4970	361F	252	38.0	53.7	38.6	4.8	4.4	5.0
G 4970 + choline	364F	290	42.7	62.8	42.9	4.6	4.5	4.9
Normal	437F	245	47.5	66.2	47.6	4.4	4.0	4.7
4970	358F	340	46.8	68.8	50.1	4.8	4.6	4.8
H 4976 + choline	421F	370	51.6	75.0	53.5	4.8	4.1	5.2
Normal	346F	368	49.9	68.5	50.2	4.7	4.3	5.4

[1] The ration contains the alcohol extract, and bone development should be normal. These rations are not described in the text.

period the chicks were sacrificed and three of the long bones were dissected out and measured.

The ideal comparison would be between three types of diets, (1) perosis-producing, (2) the same as (1) but with choline added, and (3) a diet that contains the alcohol extract and is known to prevent perosis. Unfortunately chicks of suitable weight for comparison were not available in every case. The ideal was attained in five of the trials appearing in table 5. In three of them, E, F, and H, the first two diets differed not only in content of choline, but also in the content of the water extract, no. 4080. It is uncertain whether this latter difference is the explanation, but it will be noted that in one trial, F, choline failed to bring about normal bone development. In trial G the second ration is the same as the first, excepting the presence of choline, but bone development of the chick which received choline is intermediate between the one which did not, and the one which received the alcohol extract. With the exceptions noted the data are fairly consistent and there is no doubt that choline has perosis-preventing activity. However, it remains to be seen whether it is identical with the active agent present in liver, or in other effective natural foodstuffs.

The weights of the chicks were not markedly increased by supplying choline, though the data were too few and variable for any precise distinction. When the rations are entirely adequate in all other respects the differences might be more conspicuous.

SUMMARY

1. A procedure was devised by which perosis can be produced uniformly in the chick, even when manganese is supplied in abundance.

2. This type of perosis is due to the absence of a specific organic nutrient. Choline is such a nutrient, but it was not shown that it is the only one.

3. Under the procedure followed the supply of calcium, phosphorus, manganese, iron, aluminum, or zinc was not responsible for either the incidence or degree of perosis.

4. The long bones of perotic chicks are abnormally short and thick.

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ARSENIC AND GOITER

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(Received for publication October 26, 1940)

In a recent paper Scott ('38) has collected data which provide strong circumstantial evidence of a goiterogenic role for arsenic. Arsenic through its action of combining with sulphydryl groups and therefore impairing one of the oxidation reduction systems of the body might influence thyroid activity. There are numerous reports of an antagonism between arsenic and thyroid. Hesse ('33) in one of the most recent studies found that arsenic limited the toxicity and prevented death in animals poisoned by thyroxin and thyroid. If arsenic is goiterogenic there should be a high incidence of goiter in Styria, the home of the arsenic eaters. Scott ('38) states that "the incidence of goiter, cretinism and deaf-mutism in that region (Styrian Alps) is tremendous". Furthermore there is a high incidence of goiter in Cordoba province, Argentina, where there are several endemic loci of poisoning from arsenical waters.

Arsenic is used extensively as an insecticide and although most of the spray may be removed from the surface of the food before it is eaten, much of the material eventually reaches the soil of the field in which it is used where it is a potential contaminant for any vegetation that might grow there. In the treatment of syphilis arsenic compounds play a major role and arsenic with iron is probably still used extensively as a tonic. Since the body is likely to obtain considerable arsenic from one source or another, its relation to goiter should be determined.

In a previous study (Sharpless, Pearsons and Prato, '39), rats fed a soy bean flour diet with 4.0 μg. of iodine added per 100 gm., had thyroids twice the normal size. Such rats should be very sensitive to any goiterogenic substance. This is a report of the effect of arsenic on the thyroid size and iodine content of rats fed this diet.

EXPERIMENTAL

The experimental procedures were the same as those previously described (Sharpless, Pearsons and Prato, '39). Young rats, both male and female, weighing 60 to 80 gm., were used in each experiment. Although the thyroid weight per unit of body weight is the same for both sexes, males and females were distributed evenly between the diets. The percentage composition of the basal diet was as follows: Unprocessed soy bean flour 75; sucrose 15.5; butterfat 5; dried yeast 3; sodium chloride 1; calcium carbonate 0.5. Supplements of iodine supplied as KI, arsenic acid and arsenous acid were added to this mixture as shown in table 1.

TABLE 1

Supplements to the diets

	DIET NO.					
	208	210	278	279	282	285
KI, μg. "I"/100 gm.	4.0	50.0	4.0	4.0	50.0	4.0
Arsenic pentoxide, mg./100 gm. "AS"	—	—	5.0	20.0	20.0	—
Arsenic trioxide, mg./100 gm. "AS"	—	—	—	—	—	20.0

The arsenic acid was added in water solution while the arsenous acid, being only slightly soluble, was added as a dry powder. The control animals were given the basal diet with addition of 4 μg. of iodine per 100 gm. (diet 208) or 50 μg. of iodine per 100 gm. (diet 210). The experimental animals which were litter mates of the controls, were given the same diets with the addition of arsenic.

RESULTS

The average gain in body weight which can be used as an indication of toxicity, the thyroid weight and iodine content

are shown in table 2. Since similar results were obtained in all of the control groups fed diet 208 for 7 weeks, they were combined and tabulated as one group.

Fifteen rats fed diet 278 with 0.3 to 0.4 mg. arsenic intake per day had thyroids that weighed 19 mg. per 100 gm. of body weight compared with 17 mg. for controls fed diet 208. The results were analyzed mathematically according to the method described by Dunn ('29). The analysis indicated that there were eight chances in a hundred of obtaining this difference by pure chance and therefore the difference is not significant. Though the iodine content of the glands was slightly decreased,

TABLE 2

Thyroid weight and iodine content

DIET	NO. OF RATS	LENGTH OF EXPER.	AVERAGE GAIN IN WEIGHT	AVERAGE WET THYROID WEIGHT		SIGNIFICANCE RATIO TO CONTROLS	PROBABILITY	IODINE IN THYROID	
				Total	Per 100 gm. body weight			Average total	Dry weight
		days	*gm.*	*mg.*	*mg.*		%	*μg.*	%
208	54	49	95	26	17 ± 0.4			2.1	0.033
278	15	49	95	31	19 ± 0.7	1.72	8.0	1.8	0.024
279	31	49	54	28	22 ± 0.6	4.7	0	1.1	0.018
285	14	49	81	27	17 ± 0.6	—	—	2.3	0.040
208	6	81	119	32	18 ± 1.7	—		—	—
278	6	81	112	36	19 ± 0.6	—		—	—
210	12	49	98	13	8 ± 0.4			6.6	0.166
282	15	49	76	14	10 ± 0.4	2.21	2.8	5.6	0.107

body growth was unaffected. Similar results were obtained with six rats kept on the diet for 81 days instead of the usual 7 weeks. With four times as much arsenic (diet 279) thyroids which weighed 22 mg. per 100 gm. of body weight were obtained. There is less than one chance in a million that this is fortuitous and therefore this is a significant difference. Body growth and thyroid iodine concentration were approximately 50% of the values obtained in the controls.

While the total thyroid weight was not increased, a comparison of rats of different weights can be made only on the thyroid weight per unit of body weight. A greater demand on the thyroid and consequently a larger gland would be ex-

pected in animals with the greatest growth providing the iodine content of the diet remains at the same level. According to published data, Remington's ('37) as well as our own, this is true. Therefore, if diet 279 were not goiterogenic we would have expected the thyroids to be smaller on the basis of total weight, as well as per unit of body weight, than those of rats fed diet 208. Since total thyroid weight was slightly greater and the weight per unit of body weight was significantly increased, while growth was limited, the conclusion is that arsenic can act as a goiterogenic substance.

Except that it was in trivalent form, the same concentration of arsenic as in diet 279 was fed to fourteen rats (diet 285). Thyroid weight and iodine content were almost identical with the values obtained for controls. The decreased rate of growth is not significant because the growth of litter mate controls of this group was also low. The results were unexpected and can be explained with the present data only on the basis of the insolubility of arsenous acid. Since the toxicity of arsenous acid depends upon the size of the particles (Schwartze, '22), it is probable that insufficient arsenic was absorbed to exert either a toxic or goiterogenic effect.

In order to determine whether the goiterogenic action could be prevented by iodine and also whether iodine would influence the toxicity of arsenic, 20 mg. of arsenic and 50 μg. of iodine per 100 gm. of diet were fed in diet 282.

The same amount of iodine was fed in the control diet 210. Thyroids weighing 10 mg. per 100 gm. of body weight were obtained in the arsenic-fed rats compared with 8 mg. per 100 gm. of body weight for their controls. Mathematical analysis indicated that there were approximately three chances in a hundred of this difference being fortuitous. In view of the variation that occurs in the thyroid weight, it is questionable whether this is a significant difference in weight. However, the thyroid iodine content was reduced to nearly 0.1% of the dry weight, the minimum content compatible with normal structure. Growth was approximately 80% of that of controls which was better than the 50% obtained in diet 278. Thus, arsenic was less toxic in the presence of higher iodine intake;

it caused increase in the size of the thyroid, of probable but not proven significance; it definitely reduced the iodine content and concentration of the thyroid.

DISCUSSION

These experiments show that arsenic can act as a positive goiterogenic substance and that iodine can reduce the goiterogenic action as well as the toxicity of small amounts of arsenic. The iodine requirement is increased appreciably. The total iodine content of diet 282 was approximately 55 μg. per 100 gm., nearly six times the 9.5 μg. per 100 gm. contained in our normal stock diet that gives an equivalent thyroid iodine content. Previous work has shown (Sharpless, Pearsons and Prato, '39) that rats fed soy bean flour require 20 to 25 μg. of iodine per 100 gm. of diet to produce thyroids containing 0.1% iodine. When this increased requirement is taken into consideration, the iodine requirement of rats fed diet 282 is more than twice that of rats fed the same diet without arsenic.

Can arsenic act as a positive goiterogenic agent in man? If the results obtained with rats give a direct indication, the answer is a qualified "no". They show that under ideal conditions for exerting a goiterogenic action, arsenic in nontoxic amounts has an insignificant effect. However, the results also indicate that in those areas where the iodine intake is relatively low, a goiterogenic effect can be expected if the arsenic intake is sufficient to be slightly toxic. While it is well known that people can consume huge doses of arsenic when it is eaten in large particles there is little experimental evidence to support the belief that a great tolerance for it can be acquired. Schwartze ('22) reviewed animal experiments as well as reports on the arsenic eaters and concluded that the so-called habituation to arsenic can be explained more readily on the basis of non-absorption from the intestinal tract than on the basis of an increased physiological tolerance for the poison.

The mechanism by which arsenic exerts its goiterogenic effect may be indirect, that is, by interfering with some of the normal oxidation-reduction systems. Glutathione decreases the toxicity of arsenic (Rosenthal and Voegtlin, '30). This

suggests that glutathione and possibly other sulphydryl containing compounds of the living cells are involved in a chemical union with arsenic. Barry, Bunbury and Kennaway ('28) have shown that arsenic has a retarding action on at least three oxidation-reduction systems. If by combination with the sulphydryl group or some other means, arsenic can hinder cell oxidation, then the thyroid might be stimulated by an effort of the body to compensate for lost oxidative capacity.

SUMMARY

1. Arsenic fed in non-toxic amounts (0.005% of the diet) had a slight, but not significant goiterogenic effect.

2. When the concentration of arsenic was 0.02% of the diet, growth was decreased 50%, iodine concentration of the thyroid was decreased and the thyroid weight was significantly increased.

3. Five times the minimum requirement of iodine when fed with 0.02% arsenic produced an enlargement of questionable significance, but lowered the thyroid iodine concentration.

4. It was calculated that 0.02% arsenic in the diet more than doubled the iodine requirement.

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CHEMICAL ESTIMATION OF QUALITY IN ANIMAL PROTEIN CONCENTRATES

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ONE FIGURE

(Received for publication November 4, 1940)

In an earlier report (Almquist, Stokstad and Halbrook, '35) details were given of a chemical method for the estimation of quality in animal protein concentrates. Good agreement was shown between chemical and biological data obtained on a variety of samples. In the present report it is desired to add some further results of a supporting nature.

PROCEDURE

Chemical investigations of protein concentrates were conducted as already described (loc. cit.). The biological testing procedure was also very similar to that formerly used. White Leghorn chicks were used in all tests.

The basal diet contained wheat bran 10 parts, ground wheat 20, ground yellow corn 20, corn starch 25, dried alfalfa 4, dried brewer's yeast 5, oil [1] 3, sardine oil ½, salt, plus small amounts of manganese, iron and copper 1, limestone 1½. To 90 parts of this basal ration were added the animal protein supplement in amount sufficient to provide 6 parts of crude protein, and bone meal to adjust the total bone meal content of the ration to 3 parts. The total protein level of the completed ration was close to 15% in all cases.

As a biological measure of the quality of the protein concentrate, the gain per unit of food consumed from 4 to 6

[1] Wesson.

weeks of age was selected as being more free from the influence of seasonal and other factors which may affect total gain. Chemical and biological results obtained are given in table 1. This table includes the data of three separate experiments in each of which the same standard sample of sardine meal was included. The gain per unit of feed consumed in the case of the sardine meal was 0.300 in one test and close to this figure

TABLE 1

Chemical and biological data on animal protein concentrates

SAMPLE NO.	DESCRIPTION	PERCENTAGE OF SAMPLE AS PROTEIN					PROTEIN QUALITY INDEX	GAIN PER UNIT FEED
		Total crude	Copper-precipitable	Undigestible	Phosphotungstic acid-precipitable	Hot-water-soluble		
86	Sardine meal	69.3	67.0	5.0	0.6	3.1	87.2	0.300
87	Shark meal	74.0	65.5	20.1	3.1	6.2	58.1	0.197
88	Shark meal	81.1	68.3	4.9	1.5	5.4	75.0	0.263
89	Sardine "stick," dried	68.3	35.1	1.6	8.7	28.4	29.3	....
90	Blend, 8 parts 86, 1 part 89	....	....	....	....	....	80.7 [1]	0.281
91	Menhaden "stick," dried	62.3	37.6	3.3	6.5	25.2	34.8	....
92	Blend, 8 parts 86, 1.1 parts 91	....	....	....	....	....	81.2 [1]	0.284
93	Cartilage, dried	74.8	68.9	5.9	0.0	16.0	71.4	0.248
94	Blend, 4.4 parts 86, 4.0 parts 93	....	....	....	....	....	79.3 [1]	0.260
95	Gelatin	91.9	91.9	0.0	0.0	91.9	40.0	0.150
96	Blend, 4.5 parts 86, 3.3 parts 95	....	....	....	....	....	63.6 [1]	0.237

[1] Calculated from the indices of the separate concentrates.

in the other two. In these latter two tests the gain coefficients were slightly corrected throughout by simple proportion to a value of 0.300 for the standard sardine meal.

The protein quality index was calculated for each sample as in the former report. This calculation may bear repeated explanation. It consists of subtracting from the copper-precipitable protein (table 1) all of the undigestible protein and

0.6 of the hot-water-soluble protein. To the result is added 0.4 of the phosphotungstic acid-precipitable protein, and the final sum is divided by the total crude protein of the sample and multiplied by 100. Reasons for these steps and for the coefficients were given in the earlier paper. The relation of the protein quality index of the supplements or blends to the gain per unit of feed consumed is shown in figure 1.

As discussed in detail in the former report, the chemical analysis affords an insight as to the nature of the differences in quality that exist between samples. On the other hand, a

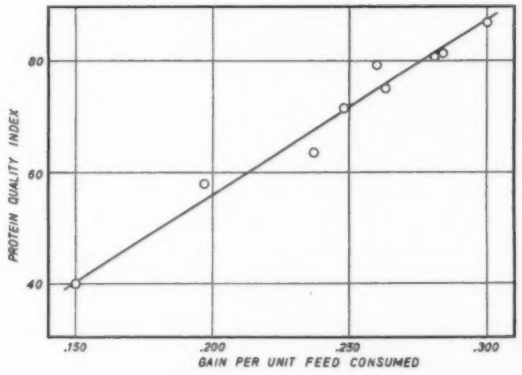

Fig. 1 Relation of protein quality index to gain per unit of feed consumed.

biological test merely demonstrates a difference without disclosing the underlying causes of the difference. For example, it is evident from the analyses that the principal point of inferiority of sample 87, as compared to sample 88, both shark meals, is a high content of undigestible protein. This is reflected in the index and also in the gain coefficient.

Dried fish meal "stick" shows a very unfavorable analysis, featured particularly by a high content of hot-water-soluble protein quite similarly for samples 89 and 91. Blends of these samples with the standard sample 86 gave lower gain coefficients in direct relation to lower protein quality indices of the blends. These results indicate that the blending of "stick"

into fish meal is likely to reduce the general protein quality of the latter.

Sample 93, cartilage, gave better results both chemically and biologically than might have been expected. A blend of cartilage with sardine meal (sample 94) was intermediate to these samples in respect to gain. The results with gelatin (sample 95) were similar to those formerly obtained (loc. cit.), while the blend of gelatin with sardine meal (sample 96) gave intermediate growth results in agreement with the calculated index.

Fish meal was used as a standard source of protein rather than the more commonly employed casein. This choice was based on the fact that casein which is deficient in arginine (Klose, Stokstad and Almquist, '38) and in glycine (Almquist, Stokstad, Mecchi and Manning, '40) is an inadequate protein for chicks. In protein test diets, the protein level is usually decidedly sub-optimal in order to insure maximum protein utilization and to insure a rigorous test of the adequacy of the protein supplement. The amino acid deficiencies of casein for chicks in these low-protein diets may produce a situation in which the casein is not a standard protein but, instead, a variable particularly dependent on the amino acid composition of the remainder of the diet.

Due to the interest which has arisen concerning the chemical method for estimating protein quality there have been some attempts to apply the chemical method to products such as vegetable protein concentrates and other types of concentrates which do not properly belong in the group made from animal flesh or skeletal tissues. Such use of the chemical method has not been investigated by us and is not, at present, recommended.

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THE UTILIZATION OF THE CALCIUM OF MILK BY ADULTS

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(Received for publication November 1, 1940)

INTRODUCTION

In the past, many nutritionists have classified milk as being an almost indispensable component of the American dietary. This high rating has been due primarily to the richness of this food in calcium. But, as has been pointed out before (Kinsman, Sheldon, Jensen, Bernds, Outhouse and Mitchell, '39) and so effectively demonstrated in studies on the availability of the calcium of spinach (e.g., Fairbanks and Mitchell, '38) the worth of a food is not determined solely by the amount of a given nutrient contained in it; the extent to which that nutrient can be utilized would also affect the rating of the food. From studies on pre-school children, it is apparent that only about one-fifth of the calcium of milk is available to them (Kinsman et al., '39). However, it is probable that they cannot make any better use of the calcium from other sources—at least, they could utilize the calcium of $CaHPO_4$ to the extent of approximately 20% (Kempster, Breiter, Mills, McKey, Bernds and Outhouse, '40). Comparable data on the adult's ability to utilize calcium are extremely meager; the man studied by Steggerda and Mitchell ('39) is the only adult who has been subjected to an experiment designed specifically to determine utilization. This dearth

[1] A portion of these data was presented by Miss Julia Dwight in partial fulfillment of the requirements for the degree of Master of Science at the University of Illinois, June, 1940.

of specific information on adults prompted the undertaking of the study reported here, and, since milk rates so highly as a source of calcium, this food was selected for the initial investigation.

EXPERIMENTAL

The general plan of the experiment was similar to that of the earlier calcium studies which have been conducted in these laboratories (Outhouse, Kinsman, Sheldon, Twomey, Smith and Mitchell, '39; Kinsman, Sheldon, Jensen, Bernds, Outhouse and Mitchell, '39), and reference to these publications should be made for detailed information concerning the preparation of the food for serving, the separation of the metabolic materials into their respective periods, etc. An exception to the usual procedure was the institution of 5-day, rather than 7-day, metabolic periods, thus necessitating the use of only five different menus in the basal dietary. Other slight deviations were the change in the quantity of milk calcium—only 175 gm. of coffee cream were allowed for each 5-day period, and the substitution, on 4 of the 5 days, of eggs and bacon or sausage for the breakfast cereal. Moreover, all foods except the vegetables were given in larger quantities. The assortment and quantity of foods are given in table 1. Throughout the experiment, vitamins A and D, in the form of a fortified halibut liver oil, were fed in quantities of, at least, 18,800 and 1880 I.U., respectively, for each 5-day period. In addition, because the dietary was almost devoid of milk, 3 to 6 gm. of a water-alcohol extract of rice-polishings were given daily.[2]

Four women and three men served as subjects for the study. The women, all of whom were members of the home economics staff, probably were considerably better nourished than the men—at least, they had habitually consumed, as milk calcium, the equivalent of 1 pint of milk daily, whereas two of the men

[2] The vitamin A and D preparation was the Hi-vi-tol sold by the E. L. Patch Company, Stoneham, Massachusetts; the rice-polishings extract was the "Galen-B" of the Galen Company, Berkeley, California.

TABLE 1

The basal diet used throughout each metabolic period

DAY I		DAY II		DAY III		DAY IV		DAY V	
Article of food	Weight	Article of food	Weight	Article of food	Weight	Article of food	Weight	Article of food	Weight
Breakfast									
	gm.		*gm.*		*gm.*		*gm.*		*gm.*
Orange juice	100	Orange juice	100	Orange juice	100	Orange juice	100	Orange juice	100
Sausage (pork)	50	Bacon	50	Cereal (cubs)	30	Bacon	50	Bacon	50
Egg	75	Egg	75	Cream	75	Egg	75	Egg	75
Cream (20%)	25	Cream	25	Toast	ad libitum	Cream	25	Cream	25
Toast	ad libitum	Toast	ad libitum	Honey	ad libitum	Toast	ad libitum	Toast	ad libitum
Honey	ad libitum	Honey	ad libitum	Butter	ad libitum	Honey	ad libitum	Honey	ad libitum
Butter	ad libitum	Butter	ad libitum	Coffee	ad libitum	Butter	ad libitum	Butter	ad libitum
Coffee	ad libitum	Coffee	ad libitum			Coffee	ad libitum	Coffee	ad libitum
Luncheon									
Pears	75	Tunafish	120	Liver sausage	2 slices	Sausage	75	Tongue	50
Bananas	75	Lettuce	40	Spiced ham	1 slice	Apples	100	Beets	100
Grapefruit	50	Pickle	15	Pork loaf	1 slice	Tomato juice	100	Lettuce	25
Lettuce	40	Green pepper	15	Lettuce	30	Bread	ad libitum	Prunes	40
Noodle soup	250	Tomato juice	100	Pickle	15	Butter	ad libitum	Bread	ad libitum
Toast	ad libitum	Prunes	40	Pineapple pulp	80			Butter	ad libitum
Butter	ad libitum	Toast	ad libitum	Pineapple juice	100				
Salad dressing	ad libitum	Butter	ad libitum	Angel food cake	½ cake				
		Salad dressing	ad libitum	Bread	ad libitum				
				Butter	ad libitum				
Dinner									
Beef	150	Ham	150	Chicken	100	Beef	150	Lamb	150
Peas	75	Beans (green)	80	Peas	85	Potatoes	125	Corn	85
Potatoes	125	Sweet potatoes	125	Potatoes	125	Onions	10	Potato	125
Pineapple pulp	80	Apricots	100	Strawberries	75	Beans (green)	80	Strawberries	100
Pineapple juice	100	Toast	ad libitum	Bread	ad libitum	Peaches	80	Biscuits	40
Toast	ad libitum	Butter	ad libitum	Butter	ad libitum	Celery salt	0.5	Butter	ad libitum
Butter	ad libitum	Coffee	ad libitum	Coffee	ad libitum	Toast	ad libitum	Coffee	ad libitum
Coffee	ad libitum					Coffee	ad libitum		

had previously existed on a low calcium dietary for several years. However, all were healthy and apparently normal. Their ages, heights and weights are given in table 2.

The experiment was so planned that the formula for the computation of percentage utilization used in the studies on children (Kinsman et al., '39) could be applied in this study on adults. Calcium was still fed at two levels of intake, but, inasmuch as a maximal value for calcium utilization could not be assured if the adults were in calcium equilibrium, both levels were designed to provide less than the maintenance requirement for calcium and, therefore, to induce negative balances. The lower of these two intakes was provided by a basal dietary; the higher one was derived from the specific food being tested—in this case, milk—which was added to the basal dietary. Obviously, if a smaller calcium deficit existed when this food was being tested, it would follow that the calcium of the test substance was being utilized, and a quantitative measure of its utilization could be secured by applying the following formula:

$$\frac{\text{Net losses during basal period} - \text{net losses during test period}}{\text{Intake during test period} - \text{intake during basal period}} \times 100.$$

The basal series preceded the milk series, and the two were separated by a 12-day interval during which time the subjects were permitted to choose their own dietaries, but large quantities of milk or $CaHPO_4$ had to be a part of it. (This short interval of liberal calcium ingestion was also in effect prior to the basal period.) The basal dietary was fed for 34 days (metabolic periods 8 through 13), and, subsequently, it was supplemented with milk for 24 to 34 additional days (metabolic periods 14 through 19). In both series (i.e., basal and milk), collections of metabolic materials were omitted during the first 4 days.

Pasteurized milk [3] was used throughout the experiment. The quantity which was fed was governed by the need to give

[3] The milk was obtained from the College of Agriculture herd which included Jersey, Guernsey, Holstein and Brown Swiss cows and had an average calcium content of 121 mg. per 100 gm.

TABLE 2

Daily calcium exchange and the utilization of milk calcium

PERIOD		INTAKE	OUTPUT Urinary	OUTPUT Fecal	BALANCE
		Subject Jd Age 22 Sex F		Weight in kg. Height in cm.	53 166
		mg.	mg.	mg.	mg.
Basal	8	251	178	184	—111
	9	234	154	128	— 48
	10	250	173	129	— 52
	11	245	182	105	— 42
	12	234	182	152	—100
	13	273	206	116	— 49
	Ave.	248	179	136	— 67
Milk	14	462	189	278	— 5
	15	455	233	228	— 6
	16	459	217	326	— 84
	17	441	233	232	— 24
	18	439	236	266	— 63
	Ave.	451	221	266	— 36

$$\% \text{ Utilization } \frac{67\text{-}36}{451\text{-}248} \times 100 = 15.3$$

PERIOD		INTAKE	OUTPUT Urinary	OUTPUT Fecal	BALANCE
		Subject Mh Age 24 Sex M		Weight in kg. Height in cm.	60 166
		mg.	mg.	mg.	mg.
Basal	8	287	110	199	— 22
	9	274	130	284	—140
	10	270	124	132	+ 14
	11	295	150	277	—132
	12	252	120	218	— 86
	13	267	99	202	— 34
	Ave.	274	122	219	— 67
Milk	14	580	118	544	— 82
	15	593	137	452	+ 4
	16	597	168	397	+ 32
	17	569	164	387	+ 18
	18	560	161	435	— 36
	Ave.	580	150	443	— 13

$$\% \text{ Utilization } \frac{67\text{-}13}{580\text{-}274} \times 100 = 17.6$$

		Subject Ws Age 22 Sex M		Weight in kg. Height in cm.	65 171
Basal	8	319	144	257	— 82
	9	332	127	304	— 99
	10	298	139	181	— 22
	11	302	131	247	— 76
	12	283	111	215	— 43
	13	322	110	276	— 64
	Ave.	309	127	247	— 64
Milk	14	608	146	440	+ 22
	15	622	148	464	+ 10
	16	602	152	496	— 46
	17	594	133	445	+ 16
	18	589	152	466	— 29
	Ave.	603	146	463	— 5

$$\% \text{ Utilization } \frac{64\text{-}5}{603\text{-}309} \times 100 = 20.1$$

		Subject Rd Age 24 Sex M		Weight in kg. Height in cm.	74 181
Basal	8	302	179	321	—197
	9	291	105	259	— 73
	10	286	131	313	—158
	11	273	121	239	— 87
	12	273	168	284	—179
	13	308	147	313	—152
	Ave.	289	142	288	—141
Milk	14	906	168	699	+ 39
	15	900	174	797	— 71
	16	879	170	746	— 37
	17	828	187	499	+142
	18	850	197	835	—182
	Ave.	873	179	716	— 22

$$\% \text{ Utilization } \frac{141\text{-}22}{873\text{-}289} \times 100 = 20.4$$

		Subject Bm Age 22 Sex F		Weight in kg. Height in cm.	55 167
Basal	8	278	243	147	—112
	9	273	228	131	— 86
	10	256	214	123	— 81
	11	244	206	103	— 65
	12	251	247	95	— 91
	13	280	223	152	— 95
	Ave.	264	227	125	— 88
Milk	14	622	223	333	+ 66
	15	618	253	332	+ 33
	16	627	290	238	+ 99
	17	573	311	372	—110
	18	473	283	232	— 42
	19	496	255	255	— 14
	Ave.	568	269	293	+ 5

$$\% \text{ Utilization } \frac{5\text{-}(\text{-}88)}{568\text{-}264} \times 100 = 30.6$$

		Subject Hb Age 27 Sex F		Weight in kg. Height in cm.	61 161
		273	146	180	— 53
		281	134	222	— 75
		268	168	203	—103
		278	121	198	— 41
		271	128	246	—103
		295	147	252	—104
		278	141	217	— 80
		568	104	452	+ 12
		582	119	534	— 71
		568	108	280	+180
		564	137	463	— 36
		572	135	330	+107
		589	137	500	— 48
		574	123	427	+ 24

$$\% \text{ Utilization } \frac{24\text{-}(\text{-}80)}{574\text{-}278} \times 100 = 35.1$$

		Subject Jo Age 42 Sex F		Weight in kg. Height in cm.	64 161
Basal	8	243	156	148	— 61
	9	237	186	164	—113
	10	225	231	150	—157
	11	226	205	109	— 88
	12	219	192	213	—186
	13	240	188	162	—110
	Ave.	231	193	158	—120
Milk	14	501	188	338	— 25
	15	494	201	297	— 4
	16	501	206	363	— 68
	17	497	275	280	— 58
	Ave.	498	218	319	— 39

$$\% \text{ Utilization } \frac{120\text{-}39}{498\text{-}231} \times 100 = 30.3$$

calcium at a sufficiently low level to insure negative balances—and thus attain maximal utilization of the milk calcium—and by the desire to feed almost enough to induce calcium equilibrium so that, subsequently, the calcium requirements of these subjects could be determined. Since the amount of calcium which these subjects lost during the basal period varied greatly, it is obvious that the amount of milk required would vary accordingly. The actual amounts fed daily were 180 gm. for Jd, 220 for Jo, 260 for Hb, Mh and Ws, respectively, and 500 for Rd; subject Bm was given 300 gm. at the beginning of the experiment, but, when it was found that she was in positive balance, the quantity was decreased to 200 gm. daily during the last two periods.

RESULTS

The daily calcium intakes, excretions and balances of the seven subjects are recorded in table 2. During the 30 consecutive days in which the basal dietary was ingested, the average daily calcium intake was 248, 264, 278, 274, 231, 309 and 289 mg., respectively, for subjects Jd, Bm, Hb, Mh, Jo, Ws and Rd; the average for the seven subjects was 270 mg. The corresponding average daily excretions of calcium were 315, 352, 358, 341, 351, 374 and 430 with an average of 360 mg.—resulting in daily net losses of 67, 88, 80, 67, 120, 64 and 141 mg. When these values are based on body weight, they represent intakes of 4.8, 4.8, 4.8, 4.6, 3.6, 4.7 and 3.9 mg. per kilogram and total calcium excretions of 6.4, 6.4, 6.0, 5.7, 5.5, 5.7 and 5.8 mg. per kilogram.

During the milk series, the average daily total calcium intakes for subjects Jd, Hb, Mh, Jo, Ws and Rd were 451, 574, 580, 498, 603 and 873 mg., respectively. Of these quantities, milk contributed 220, 318, 318, 271, 318 and 601 mg. for the subjects listed in the above order. During the first 4 weeks, subject Bm's calcium intake averaged 610 mg., over half of which (361 mg.) was contributed by milk. At these levels of intake, subjects Jd, Mh, Jo, Ws and Rd were in negative

calcium balance to the extent of 36, 13, 39, 5 and 22 mg., having excreted 487, 593, 537, 609 and 895 mg. calcium, respectively. Subjects Bm and Hb were in positive balance; they stored as a daily average, 22 and 24 mg., respectively. During the last two periods when subject Bm was given only 489 mg. of calcium, she lost an average of 28 mg. of calcium daily. (The calcium intake for subject Hb was not reduced because the alternating positive and negative balances made it impossible to ascertain, until it was too late to change, whether or not the level of intake was suitable.) On the basis of body weight, the calcium intakes were 8.7, 11.1, 9.6, 9.7, 7.8, 9.3 and 11.8 mg. per kilogram; the total calcium excretions were 9.4, 10.7, 9.2, 9.9, 8.3, 9.4 and 12.1 mg. per kilogram.

From the data for these two periods, the following values for the utilization of milk calcium can be obtained for subjects Jd, Hb, Mh, Jo, Ws, and Rd, respectively: 15.3, 35.1, 17.6, 30.3, 20.1 and 20.4%. Two values are computable for subject Bm—31.8% for the first four periods when she received 300 gm. of milk, and 27.1% for the 10 days on the reduced intake of 200 gm. milk daily; inasmuch as a 10-day period is probably not long enough to insure reliable data, the use of an average for all six periods—i.e., 30.6%—would make for greater accuracy. When this value is included, the average utilization of the entire group of seven subjects becomes 24.2%.

DISCUSSION

The two highest values reported here (i.e., 30.6 and 35.1%) were obtained for the two subjects who were in positive balance; inasmuch as these individuals were ingesting a greater quantity of calcium than was needed for equilibrium, one might raise the question as to whether or not the values represent the maximal ability of these subjects to utilize milk calcium. The question is answered for subject Bm by the fact that, when she was fed a level of calcium low enough to put her in negative balance, her computed rate of utilization was no higher than it had been when she was storing calcium.

One can, therefore, conclude that, even though she was in positive balance, she had been utilizing calcium to her maximal capacity. The same conclusion, possibly, can be drawn for subject Hb whose average calcium intakes and balances approximated, respectively, those recorded for Bm during the basal series and during the first four periods of the milk series. Such positive balances as were observed for these two subjects during the milk series—i.e., 22 and 24 mg.—undoubtedly are indicative of the storage of calcium into tissues which had been depleted during the previous weeks [4] of restricted calcium ingestion. Until the skeletal tissues had been refilled with calcium it is conceivable that calcium utilization would proceed at a maximal rate.

The seven adults of this study fall into two fairly distinct groups—those who utilized 20% or less and those who utilized 30% or more. The cause for such a grouping is difficult to identify, but it is obvious that age differences could not have been responsible. Sex differences also must be ruled out; those subjects showing the most efficient usage of calcium were women, it is true, but the one who utilized the least also was a woman. She had been accustomed to large quantities of milk for many years prior to the experiment, and this fact may have been responsible for her poor utilization of milk calcium, if Rottensten's findings ('38) on rats are applicable to man—i.e., his work would lead one to expect less efficient utilization of calcium in the well-nourished individual and extremely efficient utilization in those whose skeletal tissues had been depleted. However, the three subjects who showed the highest utilization rates were well nourished with respect to calcium. Furthermore, the two who utilized only 17.6 and 20.1% were known to have been on a low calcium dietary for at least 3 years prior to the beginning of the experiment. Obviously, the nutritional status of these subjects could not account for the differences in their capacities to utilize calcium.

[4] Immediately preceding the basal series, these seven individuals had served as subjects in a study for the determination of the utilization of the calcium of carrots; they had ingested, as an average, 446 mg. calcium daily for 39 days.

There are few studies in the literature which yield information concerning the adult's ability to utilize the calcium of milk. Steggerda and Mitchell's experiment ('39) is the only one which actually was designed to give quantitative data; their one adult subject utilized milk calcium to the extent of 20%. However, there are several other subjects mentioned in the literature who had received two different, but moderate, levels of calcium and for whom, consequently, data for utilization can be computed. Two of the presumably healthy subjects studied by Sherman and co-workers utilized milk calcium as follows: a 54 kg. woman, 23% (Sherman and Winters, '18) and subject E, 76% (Sherman, '20). The upper class Indian subject of Basu, Basak and Rai Sircar ('39) utilized 22%, while the four subjects, nos. 4, 5, 6 and 7, of Owen, Irving and Lyall ('40) utilized 71, 31, 51 and 48%, respectively, of milk calcium. Some of these data fall within the range reported in the present paper, but others are considerably in excess; however, the values observed for the subjects of Owen et al. are possibly the result of an adaptation to an extremely inadequate intake of calcium over a period of many years.

It is interesting to note that, whereas the rates of calcium utilization of the eleven children studied in this laboratory (Kinsman et al., '39) fell within the range of those reported for these seven adults, such high utilization as was observed for subjects Bm, Hb and Jo (i.e., 31, 35 and 30%, respectively) was never encountered in any of the children previously studied except during the time of lactose ingestion (Mills, Breiter, Kempster, McKey, Pickens and Outhouse, '40). This disaccharide increased their utilization of calcium 33%, as an average, and the mean of their percentage retentions—i.e., 26.6%—is comparable to the average figure for calcium utilization computed in the present study.

The wide disparity in the values obtained with these adults—and with the children previously studied for utilization of milk

calcium—is good evidence that it is not the form in which calcium occurs in a given food which determines its availability. Additional proof is found in the much more efficient utilization by babies (Wang, Witt and Felcher, '24) and the almost complete usage of the calcium of milk by rats (Ellis and Mitchell, '33). If the state of calcium (i.e., whether or not it is ionized) does determine the rate of utilization, then one would expect all species and all members of a given species to make use of identical portions of the calcium in a specific food. Obviously, the factor which governs the degree of utilization must be situated in the body, but whether it controls the absorption of calcium from the intestines or whether it regulates the deposition of the absorbed calcium into the skeletal tissue is not known. Unfortunately, there is nothing in the data on pathways of excretion (in table 2) which gives any clue concerning the mechanism involved nor the reason why these seven subjects differed in their ability to utilize calcium.

SUMMARY

Seven healthy adults—four women and three men, ranging in age from 21 to 42 years—were subjected to a calcium metabolism experiment for determining their utilization of the calcium of milk. Two different levels of calcium were fed—the lower one, supplied by a basal dietary, averaged 270 mg.; the higher one was obtained by supplementing this basal dietary with enough pasteurized fluid milk to produce a slightly negative balance. The quantity of milk which each subject received was determined by the magnitude of his calcium losses during the basal period; this quantity ranged from 180 to 500 gm. daily. The basal period lasted 34 days, and milk supplemented it an additional 24 to 34 days. On respective, daily, total calcium intakes of 248, 264, 278, 274, 231, 309 and 289 mg., the subjects were in negative balance to the extent of 67, 88, 80, 67, 120, 65 and 141 mg. During the milk period, the calcium intakes were 451, 568, 574, 580, 498, 603

and 873 mg. with resulting balances of —36, +5, +24, —13, —39, —5 and —22 mg. By relating differences in calcium intakes to the differences in the corresponding calcium losses, the following values for the utilization of milk calcium were obtained: 15.3, 30.6, 35.1, 17.6, 30.3, 20.1 and 20.4%. No explanation could be found for the division of the subjects into high and low utilizers.

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FASTING CATABOLISM AND FOOD UTILIZATION OF MAGNESIUM DEFICIENT RATS

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ONE FIGURE

(Received for publication November 8, 1940)

Symptoms and sequelae of magnesium deficiency have been discussed by Kruse, Orent and McCollum ('32, '33), Greenberg and Tufts ('38), Greenberg, Lucia and Tufts ('38), and Tufts and Greenberg ('38). Calcium deprivation also has recently been shown to lead to a definite syndrome, quite different from that due to magnesium deficiency. In some respects calcium and magnesium are physiologically interchangeable, and in others antagonistic. Whereas calcium deficiency leads to lethargy, magnesium deficiency causes tetany. The effects of calcium deficiency upon fasting catabolism, food utilization, chemical composition of the body and size of its various parts have been described in a previous paper (Kleiber, Boelter and Greenberg, '40). A parallel study on magnesium deficiency is reported in this communication.

EXPERIMENTAL METHODS

The basal diet used in this work was identical with that used in the investigation on calcium deficiency (Kleiber, Boelter and Greenberg, '40) except for the salt mixtures.[1]

[1] The quantities of pure vitamins given in table 1 of the publication by Kleiber, Boelter and Greenberg ('40) are per kilogram of food.

The salt mixture used in the magnesium-deficient food consisted of 0.73 gm. NaCl, 1.0 gm. KCl, 2.2 gm. $CaHPO_4$, and 0.32 gm. $Fe(NH_4)_2(SO_4)_2 \cdot 6\ H_2O$ per 100 gm. of basal diet, and the control salt mixture differed from this only in the addition of 1.0 gm. of $MgSO_4 \cdot 7\ H_2O$. Chemical analysis showed that the magnesium-deficient diet contained 2.06 mg. and the control 84 mg. magnesium per 100 gm. of food.

The experiments were carried out according to the following plan. Ten rats were placed on the magnesium deficient diet when they were 36 days old. They were allowed an unlimited food intake for the first 5 days, then the animals were paired according to weight and fasting catabolism, and one animal of each pair was continued on the magnesium deficient diet ad libitum, while the pair mate was fed the magnesium supplied control diet in quantities so restricted that its body weight was kept as nearly equal to that of the deficient rat as was possible.

The fasting catabolism was measured twice during the preliminary 5-day period when all rats were on the magnesium deficient food, and three times during the main part of the experiment after the rats had been paired on the deficient and control diets, respectively. The measurement of the energy metabolism was carried out at 30°C. in the same multiple respiration apparatus that was used in the previous trials on calcium deficient rats (Kleiber, '40). The age and body weights of the rats at the time of each respiration trial are given in figure 1 and table 2. At the age of 98 days the rats were sacrificed, the moist and dry weights of their entire body, as well as their hearts, livers, and thyroid and adrenal glands, were measured, and the carcasses were analyzed for fat, nitrogen, ash, and magnesium. The method for the magnesium determination was the same as that used by Tufts and Greenberg ('38).

RESULTS

Growth rate and appetite. The curve for the growth of the magnesium deficient rats, shown in figure 1, is very similar

to that found for the calcium deficient rats. The greater weight attained by the magnesium-deprived animals is readily explained by the fact that the experiment on magnesium deficiency was started on rats that were 10 days older and weighed 35 gm. more than the animals used in the calcium test. The lower curve in figure 1 shows how the appetite was affected by the magnesium deficiency. The decrease in voluntary food intake was decidedly greater and more consistent than was found for calcium deficiency.

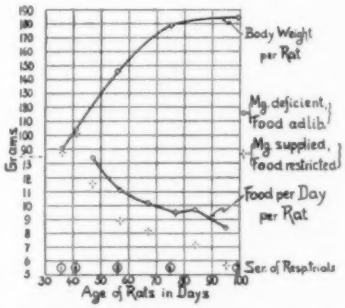

Fig. 1 Growth and food consumption curves of magnesium deficient and control rats, pair fed to maintain equal body weights.

Over the trial period of 57 days the magnesium deficient rats ate an average of 596 gm. of air dry food per animal, while the controls were given an average of only 493 gm. (83% of the food eaten by the magnesium deficient group) in order to maintain equal body weights between pair mates. At the end of the trial, when the rats were 99 days old, the average weight of the deficient rats was 184.4 $\pm$ 8.4 gm., and that of the controls 183.8 $\pm$ 7.6 gm.

Eppright and Smith ('37) have observed that rats on a control diet gained more weight than the animals fed a "low salt" diet, when both groups were fed rations that provided equal amounts of energy and protein. The results obtained by us with the technique of feeding the amount of food necessary to produce the same gain in weight, rather than giving

equal amounts of food, confirm this observation of Eppright and Smith of a reduced efficiency in food utilization for growth.

Body composition. Table 1 shows that there was no difference of great significance in the chemical composition of the carcasses of the magnesium deficient and control rats with the exception of the magnesium content which was reduced to about one-half in the deficient rats. The content of total body ash, in contrast to the result in calcium deficiency, was even slightly higher in the magnesium deficient rats. This is in agreement with the observation of Kruse, McCollum and Orent

TABLE 1

Chemical composition of magnesium-deficient rats and their pair-fed controls

All data represent the mean results for groups of five rats

	PER RAT		PER 100 GM. OF MOIST BODY OR TISSUE	
	Deficient	Control	Deficient	Control
	gm.	*gm.*	%	%
Body weight:				
Moist	184.4±8.4	183.8±7.6		
Dry	67.4±2.4	69.8±5.2	36.6	38.0
Body analysis:				
Ash	7.6±0.4	7.0±0.4	4.1	3.8
Organic matter	59.8±2.0	62.8±4.9	32.5	34.2
Fat (ether extract)	25.2±1.9	27.6±4.2	13.7	15.0
Protein (by difference)	34.6±2.0	35.2±1.6	18.8	19.2
N (Kjeldahl)	5.5±0.3	5.4±0.3	3.0	2.9
Magnesium in the body	0.029	0.055	0.016	0.030
Magnesium in the plasma			0.0013	0.0039
Magnesium in the red corpuscles			0.0028	0.0094
Calcium in the kidney			0.043	0.007

('34) and of Tufts and Greenberg ('38), namely, that there is a tendency toward increased storage of calcium in magnesium deficiency. Further evidence for the magnesium deficient state of the experimental rats is offered by the reduction observed in their levels of plasma and red corpuscle magnesium and the great increase in their kidney calcium, which are characteristic effects of magnesium deprivation in the rat (Tufts and Greenberg, '38).

Relative size of parts of body. In contrast to the effect of lack of calcium, magnesium deficiency did not affect skeletal growth. The mean distance from nose to tip of tail was 34.9 ± 0.6 cm. for the magnesium deficient and 35.0 ± 0.1 cm. for the control rats. The mean dry weight of the heart of the deficient animals was slightly greater, but the difference between the weights, 0.153 ± 0.004 gm. and 0.143 ± 0.007 gm., is not statistically significant. The mean dry weight of the liver of the deficient rats, 2.175 ± 0.152 gm., was greater than that of the controls, 1.748 ± 0.128, but the difference is on the borderline of significance, with a random probability of 5.5%. This result parallels our observations on the effect of calcium deficiency as well as those of Eppright and Smith ('37), who found that the weight of the livers of rats on a low salt diet averaged 3.68% of the body weight in mineral-deprived rats as against 2.88% in the controls. The mean dry weight of the thyroid glands was strikingly greater in the magnesium deficient than in the control rats (3.9 ± 0.4 mg. as against only 1.9 ± 0.4 mg.). The dry weight of the adrenal glands was affected in the same way, the mean weights amounting to 23.2 ± 2.3 mg. for the magnesium deficient rats as against only 14.3 ± 1.1 mg. for the controls. No such effect on these endocrine glands was observed in calcium deficiency.

Activity of rats. A. H. Smith [2] tested the activity of the magnesium-deficient rats by the same method which is described in the previous paper, and which indicated a marked decrease in voluntary activity as a result of calcium deficiency. Magnesium deficiency, it was found, did not appreciably affect the activity of the rats.

Fasting catabolism. The results of the five series of respiration trials are summarized in table 2. In the third respiration series, 15 days after the start of paired feeding, the mean catabolic rate of the magnesium deficient rats was already slightly higher than that of the controls although the dif-

[2] The authors are indebted to A. H. Smith, biochemical assistant in the division of animal husbandry at Davis, for technical assistance in carrying out the respiration trials.

ference was not significant. It became so in the fourth series; and in the fifth series, 58 days after pairing, the catabolic rate was 25% higher in the magnesium deficient rats than in the controls.

This is practically the same increase as was obtained 58 days after pairing with the trials on the calcium deficient rats (series 4, Kleiber, Boelter and Greenberg, '40). Magnesium

TABLE 2

Fasting catabolism of magnesium-deficient and control rats. Results of five pairs of rats

	SERIES OF RESPIRATION TRIALS				
	1 *	2 *	3	4	5
Age of rats in days:	36	41	56	75	99
Mean body weight in grams:					
Deficient rats	91±4	104±3	146±3	179±4	184±8
Control rats	88±1	101±3	146±2	178±5	184±8
Daily basal catabolic rate in k. cal. per rat:					
Deficient rats	17.8±0.8	20.2±0.7	23.8±1.0	25.6±1.1	26.1±1.4
Control rats	17.3±0.5	19.7±0.9	23.0±1.2	21.7±0.4	20.8±0.5
Catabolic rate per $kg^{3/4}$ † in k. cal.					
Deficient rats	107.6±3.6	109.4±4.7	100.6±4.4	93.2±4.4	93.0±6.6
Control rats	106.6±2.4	109.4±3.7	97.6±5.4	80.0±1.5	74.4±1.4
Ratio of the catabolic rate per $kg^{3/4}$ † of deficient in % of control rats	101	100	103	116	125

* In the first two trials, both groups of rats were maintained on the magnesium-deficient diet.

† Unit of metabolic body size; body weight in kilograms raised to the ¾ power.

and calcium deficiency thus have a very similar effect on catabolism even though many of their other influences on the organism are clearly very different.

Ochoa ('39) has reported that the addition of magnesium to a system consisting of dialyzed brain tissue, fumaric acid, adenylic acid, and cozymase, increased the rate of oxidation. Considering this observation one might expect a priori to find a decrease rather than an increase in the catabolic rate as a result of magnesium deficiency, which, in the present

experiments, reduced the magnesium concentration in the blood to one-third of the normal, as shown in table 1. Tufts and Greenberg ('38) have observed, however, that, even in the last stages of magnesium deficiency, the metabolically active tissues still contain almost normal amounts of magnesium. The magnesium content per unit weight of moist muscle tissue of the deficient rats amounted to 90% of that of the controls. The increase in the catabolic rate observed in magnesium deficiency may thus be related to an increase in the breakdown of tissue because of an inability to secure sufficient magnesium out of intact tissues after the stores in the bones have been depleted. The same reasoning may be applied to calcium deficiency since Boelter and Greenberg ('41) observed that the calcium content per unit weight of muscular tissue of calcium deficient and control rats was essentially the same.

A major difference between calcium and magnesium deficiency with regard to the catabolic relations is the hypertrophy of the thyroid and adrenal glands resulting from magnesium deficiency, but absent in calcium deficiency.

Magnesium deficiency and food utilization. The five magnesium deficient rats gained 80 gm. of body weight per rat in 57 days on 596 gm. of air dry food, while the controls gained 83 gm. per rat on 493 gm., or on 103 gm. less food than their deficient pair mates. This difference of 103 gm. of food represented 530 kilocalories of chemical energy. With this smaller energy intake the control animals gained 2.5 gm. body fat and 0.6 gm. body protein more than the deficient rats, an extra gain of 26 kilocalories. The deficient rats thus wasted 556 kilocalories more energy per animal in the 57 days of trial than did the controls. This extra waste amounts to 9.8 kilocalories per rat per day, or to 18.5% of the mean energy content in the 10.4 gm. of food (53 kilocalories) consumed daily on the average by a deficient rat.

The magnesium supplied controls consumed 19.7 gm. less protein than their deficient pair mates and, moreover, they gained 0.6 gm. more body protein per rat than the deficient

rats. Consequently, the deficient rats wasted 20.3 gm. more protein than the controls in the 57 days, or 0.36 gm. of protein per rat per day. This extra protein waste amounts to 18% of the mean daily intake of 1.99 gm. of food protein. Magnesium deficiency increases the protein waste to about the same extent as it increases the waste of food energy.

The rate of fasting catabolism of the magnesium deficient rats was greater than that of the controls by 5.3 kilocalories per rat per day at the end of the experiment (fifth series) and was considerably less in the earlier series. The increased rate of fasting catabolism, therefore, can account for not more than half of the extra waste of 9.8 kilocalories of food energy per day per rat on the deficient diet.

The decrease in total efficiency of food utilization in magnesium as well as in calcium deficiency results therefore not only from a higher basal metabolic rate but also from a decrease in the partial efficiency of food utilization,[3] namely, a greater loss of unoxidized material in feces and urine, or a higher calorigenic action, or a combination of these two effects.

SUMMARY

1. Five rats fed a magnesium deficient diet gradually lost appetite and, after 60 days on this regime, ceased to grow. Littermate controls could be kept at the same weight as the deficient rats by restricting their intake of magnesium-supplied food to 83% of the consumption of the deficient rats.

2. The magnesium content of the carcasses of the deficient rats was only one-half of that in the control rats, but there was no significant difference between the water, ash, fat, and protein content of magnesium deficient and control rats.

3. Body length was not affected by magnesium deficiency. The dry weights of the heart and liver were slightly greater,

[3] Total efficiency of energy utilization is defined as the quotient $\frac{\text{Total gain of body energy}}{\text{Total energy of food consumed}}$; the partial efficiency as the quotient $\frac{\text{Change in gain of body energy}}{\text{Corresponding change in energy of food consumed}}$.

and those of the thyroid and adrenal glands significantly greater in the magnesium deficient animals than in their magnesium supplied controls.

4. The rate of fasting catabolism of the magnesium deficient rats progressively increased in comparison to the rate of the magnesium supplied controls. The catabolic rate of the deficient rats amounted to 125% of the rate of the controls, 57 days after the pairing of the rats.

5. Magnesium deficiency decreased the efficiency of energy and protein utilization to approximately the same extent, causing an extra waste of 18% of the intake. The increased rate of fasting catabolism is not sufficient to explain the extra waste of energy by the magnesium deficient rats. It can therefore be concluded that magnesium deficiency causes an increased loss of unoxidized material in the excreta, or increases the calorigenic action of the food.

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THE RATE OF ABSORPTION OF VARIOUS FATTY ACIDS BY THE RAT [1]

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(Received for publication November 8, 1940)

In an earlier report from this laboratory (Deuel and Hallman, '40), it was pointed out that a marked variation obtains in the rate of absorption of the various synthetic triglycerides composed of short-chained fatty acids. Triacetin and tributyrin were the most rapidly absorbed of any of the natural or synthetic fats investigated while tricaproin and tricaprylin were somewhat more slowly utilized. On the other hand, the synthetic fats composed of the odd-chain fatty acids disappeared from the intestine at a rate only approximately 50% of the comparable triglycerides having fatty acids with an even number of carbon atoms. Thus, tripropionin, trivalerin and triheptylin all exhibited a markedly retarded absorption rate.

There are two possibilities which may be advanced to explain this divergence. In the first place it is conceivable that the pancreatic lipase may act preferentially on the even-chain triglycerides, resulting in a much accelerated rate of hydrolysis with these compounds. Since there seems to be general agreement that saponification of the triglyceride molecule must precede absorption, such a variation would offer a satisfactory explanation for the differences in absorption rate we have already noted. However, there are no in vitro experiments which support these contentions; in fact such studies by two groups of workers show widely different

[1] Some of this work was supported by a research grant from Best Foods, Inc.

results when the enzymatic hydrolysis is followed under somewhat different conditions (Balls, Matlack and Tucker, '37–'38; Weinstein and Wynne, '35–'36).

A second explanation of the divergent absorption rates of the various synthetic triglycerides may be in the differences in the speed with which the liberated fatty acids leave the gut. That the intestinal mucosa may exert such selectivity in absorption with different fatty acids does not seem difficult to accept since we know that such variations may occur between such closely related hexoses as glucose and mannose (Cori, '25).

In order to test the second possibility we have followed the rate of absorption of the fatty acids from acetic to tridecylic in the fasting rat.

METHODS

In all the tests recorded in table 2 as well as most of the 1-hour tests summarized in table 3, the fatty acids were fed in doses of 100 mg. per 100 sq.cm. of surface area to female rats previously fasted for 48 hours. In the tests with the higher fatty acids the experiments were also carried out for 3-hour intervals in which case the fatty acids were administered at a level of 200 mg. per 100 sq.cm. Surface area was calculated from the fasting body weight by the formula of Lee ('29). We have shown earlier that the most satisfactory basis for comparison of the rate of absorption of fats is on the basis of surface area (Deuel, Hallman and Leonard, '40).

The lower fatty acids (acetic to heptoic) were fed in 20% aqueous solutions as their sodium salts which had been adjusted to a pH of 7.40. Because of difficulty in obtaining sufficiently concentrated solutions of the soaps of the higher fatty acids, they were fed as the free acids. Because of the weakness of these acids, this could be accomplished without any apparent injury to the rats. When the animals were sacrificed at the end of the absorption period, the intestines appeared normal from a gross inspection except in the tests

with caprylic acid where a considerable mucous secretion had occurred in the stomach.

At the conclusion of the absorption tests, the rats were anesthetized with amytal and the intact intestine removed as described earlier (Deuel, Hallman and Quon, '39). For the removal of the fatty acids which were fed as the salts in water solution, the intestines were flushed with 200 cc. of cold water directly into 800 cc. Kjeldahl flasks; 5 cc. of 50% sulfuric acid was added, a spoonful of a talc-pumice mixture and the acids were distilled into an excess of standard sodium hydroxide using an ordinary Kjeldahl apparatus. The distillation was continued until the first appearance of SO_2 fumes was noted in the distilling flask; the flask was immediately disconnected. The amount of excess alkali was determined by titration with acid using phenolphthalein.

In the case of acetic acid it was necessary to modify the procedure by using repeated distillations. To 150 cc. of the gut washings, 5 cc. of 85% phosphoric acid was added, the mixture was boiled until 135 cc. of distillate was collected; 50 cc. of distilled water was added to the distilling flask through an opening in the distilling bulb and 50 cc. was again distilled; this was repeated three or four additional times until the acetic acid was completely distilled. An all-glass distillation apparatus with ground glass connections was employed.

For the recovery of the higher fatty acids which are not distillable with steam, the gut was flushed with 70 cc. of petroleum ether. This procedure was found quite satisfactory for removal of undigested triglycerides and fatty acids in our earlier studies. After drying the petroleum ether extract with anhydrous sodium sulfate, it was filtered into glass stoppered flasks and the residue washed five times with 15 cc. portions of petroleum ether. The washings were added to the original filtrate and the free fatty acid was titrated with standard sodium hydroxide using phenolphthalein after the addition of sufficient isopropyl alcohol to bring about a homogenous mixture with water.

When the intestines were removed immediately after the discharge of known amounts of acids directly into the stomach, and the gastrointestinal contents removed by the procedures outlined above, the recoveries noted in table 1 were obtained. These values have been corrected for the control levels obtained on the intestinal contents of fasted rats which received no fatty acids. Similar corrections are applied to the titrations obtained in the absorption tests (tables 2 and 3).

TABLE 1

The recovery of fatty acids from the gut contents of female rats killed immediately after the administration of a water solution of the sodium salt (I and II) or after the administration of the free fatty acid (III)

FATTY ACID	NO. OF TESTS	BODY WEIGHT	FATTY ACID IN MG.			
			Fed	Recovered		
				Total	Corrected [1]	Per cent [2]
Procedure I (Flushing intestine with 150 cc. H_2O; repeated distillations)						
Acetic	10	114	213	212	203	95.4±0.8
Procedure II (Flushing intestine with 200 cc. H_2O; single distillation)						
Propionic	10	123	239	223	210	87.8±0.8
Butyric	10	128	229	225	210	91.8±0.9
Valeric	10	137	230	217	200	87.0±1.5
Caproic	11	154	258	245	225	87.5±1.2
Heptoic	12	124	226	222	200	88.4±1.5
Procedure III (Flushing intestine with 70 cc. petroleum ether; titration of extract)						
Caprylic	10	86	146	139	137	94.0±1.0
Nonylic	10	75	152	135	133	87.5±1.1
Capric	13	100	182	174	171	93.4±1.0
Undecylic	17	129	215	198	195	91.1±1.2
Lauric	12	126	199	202	199	99.8±1.3
Tridecylic	10	124	192	186	183	95.3±0.9

[1] Based on correction factors for the control fasted rats as follows: Procedure I. An average of ten experiments gave a titration value of 1.59 cc. of 0.1 N NaOH. Procedure II. An average of twenty-two experiments gave a titration value of 1.70 cc. of 0.1 N NaOH. Procedure III. An average of fourteen experiments gave a titration value of 0.16 cc. of 0.1 N NaOH.

[2] Including standard error of mean $= \sqrt{\frac{\Sigma d^2}{n}} \; / \; \sqrt{n}$

d = deviation from mean.

n = number of observations.

TABLE 2

The absorption during a 1-hour period of the distillable fatty acids fed as sodium salts in quantities of 100 mg. per 100 sq. cm. to fasting female rats

FATTY ACID FED	NO. OF EXPTS.	WEIGHT BODY	SURFACE AREA	FATTY ACID IN MG.				
				Fed	Recovered		Absorbed	
					Total	Corrected [1]	Total	Per 100 sq. cm. per hr. [2]
		gm.	*sq. cm.*					
Acetic	10	126	229	229	159	167	62.0	26.6±1.0
Propionic	16	155	257	257	176	200	56.5	21.4±1.2
Butyric	18	155	257	257	142	155	101.9	39.7±1.5
Valeric	11	160	263	253	166	190	62.2	23.3±1.9
Caproic	11	140	241	243	132	150	91.2	38.0±1.7
Heptoic	20	151	252	253	165	187	65.6	25.8±1.5

[1] Correction based on average recovery given in table 1.

[2] Including standing error of mean as in table 1.

TABLE 3

The absorption of the higher fatty acids when fed as such to fasting female rats

FATTY ACID FED	NO. OF EXPTS.	LENGTH OF PERIOD	BODY WEIGHT	SURFACE AREA	FATTY ACID IN MG.				
					Fed	Recovered		Absorbed	
						Total	Corrected [1]	Total	Per 100 sq. cm. per hr. [2]
		hr.	*gm.*	*sq. cm.*					
Caprylic	23	1	102	199	174	91	97	76	37.3±1.5
	10	3	132	234	469	136	145	324	46.0±1.7
Nonylic	24	1	136	238	240	137	157	83	34.4±2.3
	23	3	146	248	500	222	254	246	32.8±1.3
Capric	22	1	97	193	177	126	140	37	19.2±1.5
	11	3	124	226	452	280	300	153	22.6±2.1
Undecylic	15	1	127	229	229	163	180	49	21.3±3.4
	9	3	126	228	458	284	312	146	21.4±0.5
Lauric	10	1	131	233	232	226	226	6	2.7±1.2
	10	3	126	228	457	429	430	27	3.8±0.7
Tridecylic [3]	15	1	120	221	222	160	175	47	20.8±3.2

[1] Corrected for recovery based on average recovery given in table 1.

[2] Including standard error of mean calculated as in table 1.

[3] Diarrhea occurred in sixteen of eighteen experiments carried out over a 3 hour period. The successful tests were not considered valid.

The fatty acids with the exception of acetic acid were chemically pure products,[2] the purity of which was established by determination of the acid equivalent.

RESULTS

The average results of the absorption tests are summarized in tables 2 and 3. Table 2 records the tests on the distillable fatty acids while table 3 lists the experiments on the higher fatty acids. The statistical evaluation of the rates of absorption of acetic, butyric, caproic, and caprylic acids when compared with the corresponding odd-chain fatty acids is given in table 4 on the basis of the Fisher "t" method ('34).

TABLE 4

The statistical evaluation of mean differences based on the Fisher "t" method

FATTY ACID	NEXT LOWER ACID		NEXT HIGHER ACID	
	"t" calculated	Significant value for "t"[1]	"t" calculated	Significant value for "t"[1]
Acetic	....	....	2.83	2.78
Butyric	9.19	2.75	6.57	2.76
Caproic	5.47	2.82	4.98	2.75
Caprylic (1 hr.)	5.23	2.75	1.01	2.75
Caprylic (3 hr.)	....	....	5.59	2.75

[1] Based on a "P" value of 0.01 (chance of difference in results being due to error in sampling 1 in 100).

DISCUSSION

The more rapid rate of absorption which we have noted earlier for the triglycerides composed of the short-chain fatty acids with an even number of carbon atoms as contrasted with those having an odd number of carbons apparently is related to a difference in the speed of absorption of the fatty acids themselves. Thus, the rates found for the absorption of acetic, butyric, caproic and caprylic acids are in each case considerably higher than those for the corresponding odd-carbon acids. Such differences are in every case highly signifi-

[2] The lauric acid was kindly furnished by Dr. Samuel Lepkovsky, of the Eastman Kodak Co. Glacial acetic acid of Baker and Adamson was used.

cant when based on the Fisher "t" method (table 4) except in the comparison of caprylic and nonylic acids. Although the differences in rate observed in the latter case are not significant in the 1-hour tests, the variation in the experiments which lasted 3 hours are highly significant.

The rate of absorption of the distillable fatty acids under the present experimental conditions is somewhat less than that observed earlier for the corresponding triglycerides but the proportionate rate is similar except with sodium acetate. This difference would seem to be traceable either to the smaller dose employed (100 mg. instead of 300 mg. per 100 sq.cm.) or to the variable effect of water solution as compared with the fat itself. The presence of the sodium ion may alter to some extent the permeability of the intestinal mucosa. Although the results with sodium acetate are significantly higher than those found with sodium propionate, they show a much slower rate than was expected from the experiments with triacetin where the most rapid absorption rate obtains. This may possibly be due to the irritant action of the sodium acetate which tends to prevent its absorption because we have found with dogs that it may act as a violent cathartic (Deuel and Milhorat, '28). However, such catharsis was not observed in the present tests.

Capric, undecylic and tridecylic acids are absorbed at a uniform rate of approximately 20 mg. per 100 sq.cm. per hour. However, they disappear at a rate considerably below that for caprylic acid and somewhat below that of nonylic acid. Essentially similar results were noted in the 1- and 3-hour tests which would indicate a constancy in the rate of absorption.

It is also significant that the lower rate in absorption obtains with those fatty acids which are insoluble in water. Because of their insolubility, the rate of formation of the fatty acid-bile salt complex may be a limiting factor in determining the speed of absorption; on the other hand with the shorter chain acids which are soluble in water, absorption should not require the formation of this complex.

The higher acids which show the lower rate of absorption are also those which give typical soaps on neutralization with alkali. However, unpublished results [3] indicate that only minimum amounts of the hydrolyzed products may be in the form of soaps. After the administration of hydrogenated cottonseed oil, the pH of the intestine was found to be approximately 7.0. This would be impossible if soap were present in large amounts since it can be buffered only by the fatty acid which is present in solution.

On the other hand lauric acid is absorbed very slowly; less than 5% (27 mg.) of that administered left the gastrointestinal tract over a 3-hour period. The slow rate of absorption may be related to the fact that it is solid at body temperature. At the termination of the absorption test, the acid which was fed in the melted state was found to have solidified in the stomach. On the other hand, trilaurin which melts at a temperature approximately 3° higher, was absorbed to the extent of 216 mg. in a 3-hour test or 22% of that fed. Although this was the lowest value noted for any triglyceride studied, it is more than five times the rate which we have found for the free acid. One would expect, however, that the lauric acid when fed as a component of a natural fat would be much more rapidly absorbed. Here the separation of solid acid in the stomach would be avoided either by solution in other triglycerides which are liquid at body temperature or because it exists as a component of a mixed triglyceride.

SUMMARY

A comparison has been made in the rate of absorption of the fatty acids by the rat from acetic to tridecylic acids inclusive. The distillable fatty acids (acetic to heptylic) were fed as the sodium salts while the higher homologues were administered as the free acids.

[3] Experiments of J. W. Mehl, L. F. Hallman and H. J. Deuel, Jr.

Butyric, caproic, and caprylic acids were absorbed the most rapidly. Propionic, valeric and heptoic acids disappeared from the intestine much more slowly while nonylic acid occupied an intermediate position. The wide discrepancy which we have noted earlier in the rate of absorption of the triglycerides composed of even- and odd-chain fatty acids may be explained on the basis of differences in removal of the component fatty acids from the gut.

The absorption of sodium acetate was much slower than might be expected from the earlier results on triacetin although the rate was greater than for sodium propionate. Capric, undecylic and tridecylic acids were absorbed at rates approximating 20 mg. per 100 sq.cm. per hour as determined in experiments of 1 and 3 hours in duration. Only minimal quantities of lauric acid left the gastrointestinal tract over a 3-hour period. The slow speed of absorption may be partly ascribed to its solidification in the stomach; however, trilaurin which has a higher melting point was shown earlier to be absorbed much more rapidly.

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BASAL METABOLISM AND HEAT LOSS OF YOUNG WOMEN AT TEMPERATURES FROM 22°C. TO 35°C.

CLINICAL CALORIMETRY NO. 54

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FIVE FIGURES

(Received for publication October 17, 1940)

INTRODUCTION

In 1939 Hardy and Milhorat published a preliminary report of calorimeter studies on three normal women showing that these women responded to warm environments by a drop in basal metabolism that had not been found in the two men studied under similar conditions. They called attention also to several other differences in heat loss, skin temperature, vaporization, and the conduction of heat through the skin. The results of observations on seven women were discussed briefly by Hardy and Du Bois ('40). This present report contains the complete calorimetric study of eight female subjects and discusses the points of difference between men and women.

Max Rubner in 1890 and 1902 described two forms of temperature regulation, (1) a "physical regulation" through increased vaporization in the warm zone and shivering or other forms of activity in the cold zone, and (2) a "chemical regulation" which changes heat production without muscular movement (or activity of the sweat glands). There seems to be no

doubt that chemical regulation exists in animals and it is well discussed by Giaga ('38). F. G. Benedict ('38) found that the "zone of thermic neutrality," the zone of lowest basal metabolism, was narrow in the case of the dog, pigeon, and mouse, and extremely wide in the case of the goose, steer, cow and sheep. In most species this zone is not far from 28°C. There has been no satisfactory evidence as to the nature of the substance or mechanism which causes a high basal metabolism in the cold and very warm zones, or, perhaps we should say, the low metabolism in the neutral zone. In the cold zone there may, of course, be some slight shivering or tension of muscles that cannot be detected by ordinary means. Burton and Bronk ('37) have described an electrical method of detecting the onset of shivering in animals which should be of use in future studies of this type. There is as yet no real evidence of "chemical regulation" in men. Hardy and Milhorat apparently were the first to demonstrate that it exists in women.

There have been several reports of slight differences in metabolism with the season of the year but there are many influences at play and the results are conflicting. Martin ('30) in a careful study of his own basal metabolism during a voyage from England to India noted a drop of 12% a few days after entering the Red Sea and a return to the previous level when the weather was cooler. Eleanor Mason ('34) who studied a group of women in India and again in England or the United States noted that most of them gave results about 5% higher in the temperate zone. In both of these cases there was time for a slow adjustment and perhaps changes in the food. In our studies the period of adaptation was only 2 to 3 hours.

There have been a good many studies (McConnell, Yaglou and Fulton, '24) of the effects of environmental temperature on the metabolism of men, but very few on women (Hick, Keaton, Glickman and Wall, '39). Many of the investigations have been performed when the metabolism was so far above basal that many of the physiological reactions were obscured.

Wiley and Newburgh ('31) gave the average curve of a large number of experiments on one normal man. When nude there was little or no change in the basal metabolism from about 25° to 36°C.; when clothed the range of level metabolism was from 18° to 34°C. Hardy and Du Bois ('38 a) using the respiration calorimeter of the Russell Sage Institute of Pathology found a level basal metabolism in two normal men from 23° to 34.5°C. The experimental conditions used in that study were duplicated for this present report on women. Quite recently Albagli ('39) has published in Brazil an excellent monograph reviewing the influence of climate on basal metabolism, expressing the belief that most of the differences which have previously been ascribed to climate and race are due to differences in nutrition. He publishes a series of studies on thirteen young men exposed to temperatures between 14° and 39°C. Most of the naked men showed little change in metabolism between 23° and 34°C. The clothed men had a level zone between about 18° and 34°C. If his results are plotted there is a slight indication of a minimal metabolism between 24° and 32°C. and a rise of 2 or 3% between 22° and 24°C., and 32° and 34°C.

Winslow, Herrington and Gagge ('37) have performed many careful experiments on young men sitting in a chair after food had been ingested, and observed an average metabolism about 33% above basal. The men when naked showed slight change in heat production over a range of 20° to 39°C. "operative temperature," an index which takes into account not only the temperature of the air but also the radiant temperature of the walls. Their normal men, lightly clothed, had a level metabolism between 20° and 39°C. In general, the literature indicates that men show an almost uniform level of metabolism at air temperatures between 23° and 34°C.

It has long been known that women have a lower basal metabolism than men but the estimates as to the average difference have ranged from 7 to 15%. Perhaps this comparison is due to the fact that most of the tests on women have been performed at a temperature somewhere in the range of falling

metabolism demonstrated by Hardy and Milhorat. Others have surmised differences between the sexes in thermal response. Oppel and Hardy ('37) could find no differences in their studies of the thermal sensitivity of the skin.

METHOD

The experimental method was essentially that used for the study of the male subjects and has been described in detail in previous publications (Hardy and Du Bois, '38 b). Some additional procedure was desirable in the present study and the routine of an experiment is briefly described. The subjects were selected after a general physical examination. Dates of menstruation were recorded although experiments were carried out regardless of this factor, excepting the first day of menstrual flow. The subject arrived at the laboratory in the postabsorptive state, having taken special care to do little or no walking in transit to the laboratory. She sat quietly in indoor clothing in the calorimeter room, which had been set to the experimental temperature the day before. This period lasted for at least 1½ hours before the beginning of the experiment. The nurse was then called and assisted in taking surface temperature measurements as the subject undressed. The nurse also weighed the subject, adjusted the rectal thermometer, and placed the subject in the calorimeter. (In this connection the authors wish to express their appreciation of the enthusiastic cooperation of Miss R. H. Roberts and her nursing staff.) Following the sealing of the calorimeter there was a preliminary period of an hour, at the end of which the surface temperature was measured by the subject. The next period was used to measure the basal metabolism and after the end of the period the surface temperature was again measured. The mean of these values was considered the average skin temperature during the basal period. There followed either another basal period or a period during which some agent (such as chill, alcohol, exercise, etc.) was studied. At the end of the experimental period the subject's blood pressure was measured.

Eight subjects have been investigated. They were all highly intelligent and carried through the simple laboratory routine without difficulty. Information in regard to diet, etc., could in all cases be depended upon. Body types represented by this group of subjects were essentially normal but included tall and slender as well as short and heavy builds.

RESULTS

The experimental data on three individual subjects are recorded in table 1. The experiments are listed in order of increasing calorimeter temperature, and in the first part of the table are recorded the data relating to heat production. In the latter part are the data relating to heat loss.

1. Basal metabolism. At least 1 and often 2 hours of the experimental period were used for measurements of heat production in the basal state. Care was taken at the colder temperatures that no shivering or tenseness from chilling occurred during these periods. With subject no. 6, it was not possible to obtain an experimental period completely free from tenseness and slight shivering except in the very warm temperatures. In all warm experiments it was necessary to end the period between bursts of sweat rather than at a specified time as the slightest movement of the subject was sufficient to raise the pressure in the calorimeter above that of the barometer. The irregularity of vaporization made it difficult to obtain good respiratory quotients at high temperatures. So far as could be judged from observation and from talking with the subject, the periods specified as basal were intervals during which the subject lay on her back motionless but awake.

The changes in basal metabolic rate for the group are shown in figure 1. In contrast to the male subjects previously studied, whose basal metabolic rates were constant throughout this range of temperature, the women, with the exception of no. 3, all showed a definite change in their basal metabolism in the warmer environments. For temperatures between 22°C. and 27°C. the average level of metabolism was fairly constant (between 31 and 38 cal./hr./M^2); between 27°C. and 32°C.

TABLE 1

Representative data obtained from three women lying nude under basal conditions in the calorimeter

SUBJECT; DATE; REMARKS	END OF PERIOD	CO_2	O_2	RQ	H_2O	URINARY N PER HOUR	HEAT PRODUCED	CALORIMETER TEMPERATURE	% RELATIVE HUMIDITY	RECTAL TEMPERATURE	HEAT ELIMINATED	SKIN TEMPERATURE Time	SKIN TEMPERATURE °C.
		gm.	*gm.*		*gm.*								
No. 1 Feb. 2, 1938	10:33									37.22		9:45	30.4
Period I Basal	11:37	23.0	19.8	0.86	27.1	0.53	66.1	23.14	23	37.00	87.8	10:20	30.7
												11:40	30.3
No. 1 Feb. 9, 1938	10:45									37.40		9:55	31.9
Period I Basal	11:35	18.1	15.4	0.87	23.3	0.42	51.8	23.27	25	37.19	69.1	10:31	31.6
												11:42	30.6
No. 1 Jan. 19, 1938	10:54									37.18		9:50	32.4
Period I Basal	11:54	20.3	18.0	0.83	24.8	0.54	59.8	25.20	20	37.02	72.2	10:40	31.6
												12:00	31.5
No. 1 Jan. 12, 1938	11:20									37.35		10:10	32.9
Period I Basal	12:20	20.9	18.5	0.83	28.9	0.42	61.8	26.88	20	37.30	66.5	11:00	32.5
												12:25	32.3
No. 1 Mar. 11, 1938	10:42									36.97		9:45	33.6
Period I Basal	11:42	18.5	15.7	0.87	28.7	0.36	53.0	30.30	20	36.96	53.8	10:30	34.3
												11:45	34.4
No. 1 Mar. 2, 1938	10:53									37.50		10:00	34.6
Period I Basal	11:53	19.8	16.8	0.87	38.8		56.5	33.70		37.61	47.5	10:35	35.7
Period II Basal	12:53	20.7	20.2	0.74	56.3	0.35	66.2	33.77		37.73	56.0	12:00	36.1
												1:00	35.6
No. 2 Mar. 25, 1938	11:12									36.94		10:50	30.8
Period I Basal	11:32	6.9	4.1	1.33	6.2	0.27	14.1	23.98	20	36.95	22.4	11:35	30.6
												11:45	30.7
No. 2 Jan. 21, 1938	11:20									36.94		9:50	32.5
Period I Basal	12:20	19.0	16.7	0.83	23.4	0.29	55.8	24.87	21	36.75	66.7	10:30	32.1
												11:05	31.6
No. 2 Jan. 14, 1938	10:52									37.27		9:45	32.5
Period I Basal	11:52	18.9	16.7	0.83	26.1	0.31	55.9	26.81	20	37.14	63.1	10:30	32.6
												12:10	32.1
No. 2 Feb. 16, 1938	10:30									36.98		9:40	31.7
Period I Basal	11:30	18.8	15.6	0.89	26.5	0.28	52.8	28.17	20	36.94	52.1	10:19	32.4
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Period I Basal	11:40	17.2	15.2	0.83	26.3		50.6	28.21	20	36.96	53.4	11:45	32.8
Period II Basal	12:40	18.6	17.5	0.77	25.7	0.38	57.8	28.38	16	37.00	55.1	12:45	32.6

	Period I Basal	11: 30	18.8	15.6	0.89	26.5	0.28	52.8	28.17	20	36.98 36.94	52.1	9: 40 10: 19 11: 34	31.7 32.4 32.6
	Period I Basal	11: 40	17.2	15.2	0.83	26.3		50.6	28.21	20	36.96	53.4	11: 45	32.8
	Period II Basal	12: 40	18.6	17.5	0.77	25.7	0.38	57.8	28.38	16	37.00	55.1	12: 45	32.6
No. 2	Feb. 18, 1938	10: 45									36.86		9: 52	33.0
	Period I Basal	11: 45	15.8	13.3	0.87	23.7	0.24	45.0	29.82	19	36.80	47.9	10: 32 11: 51	34.0 33.8
No. 2	Mar. 18, 1938	10: 49									36.74		10: 35	34.3
	Period I Basal	11: 49	16.3	12.8	0.96	29.0		43.8	31.13	20	36.70	42.8	11: 55	34.6
	Period II Basal	12: 49	16.2	13.8	0.87	47.3	0.30	46.5	31.25	17	36.71	56.1	12: 54	34.6
No. 2	Feb. 25, 1938	10: 42									36.80		9: 45	34.4
	Period I Basal	11: 42	16.6	13.5	0.91	28.9	0.29	45.9	32.02	17	36.80	41.6	10: 30 11: 45	34.9 35.0
No. 2	Mar. 4, 1938	10: 48									37.07		9: 50	33.7
	Period I Basal	12: 00	18.9	16.5	0.84	69.2	0.25	55.4	35.52		37.21	52.4	10: 35 12: 05	35.8 35.7
No. 2	Mar. 9, 1938	10: 48									37.23		9: 45	34.2
	Period I Basal	11: 48	16.8	16.1	0.75	81.2		53.2	35.89	26	37.30	52.1	10: 29	35.8
	Period II Basal	12: 49	18.8	15.5	0.89	88.3	0.18	52.8	35.75	30	37.30	55.2	11: 55 12: 53	35.5 35.8
No. 3	Mar. 14, 1939	10: 45									36.77		9: 43	32.4
	Period I Basal; cold at end	11: 41	16.7	14.0	0.87	24.1	0.44	47.1	24.59	26	36.66	67.7	10: 34 11: 45	31.6 30.5
No. 3	Mar. 7, 1939	10: 40									36.61		9: 45	32.1
	Period I Basal	11: 40	17.9	15.5	0.84	23.8	0.67	51.2	25.98	24	36.57	65.0	10: 26 11: 47	32.3 31.8
No. 3	Feb. 28, 1939	10: 32									36.83		9: 33	33.6
	Period I Basal	11: 12	11.8	9.2	0.94	17.9	0.72	30.8	26.00	28	36.71	44.9	10: 16 11: 17	32.3 31.8
No. 3	Jan. 24, 1939	10: 30									36.81		10: 13	32.4
	Period I Basal	11: 30	18.6	15.9	0.85	32.8	0.65	52.8	27.97	25	36.88	55.5	11: 38	32.7
No. 3	Feb. 7, 1939	10: 36											9: 31	33.4
	Period I Basal	11: 36	17.5	14.7	0.87	33.6	0.28	49.6	29.84	25		50.0	10: 19 11: 42	33.5 34.1
No. 3	Feb. 14, 1939	10: 19									37.12		9: 26	34.4
	Period I Basal	11: 19	17.5	15.5	0.82	35.7	0.35	51.7	31.85	25	37.03	48.2	10: 08 11: 26	35.1 35.2
No. 3	Feb. 21, 1939	10: 25									37.24		9: 24	35.0
	Period I Basal	11: 25	18.4	13.6	0.98	64.4		46.1	33.98	33	37.19	49.9	10: 05	35.2
	Period II Basal	12: 25	17.3	16.1	0.78	66.4	0.55	52.7	33.88	29	37.18	50.3	12: 29	35.2

the metabolic rates of all subjects, excepting no. 3, decreased to an average of 30.9 cal./hr./M². In table 2 are given the

TABLE 2

Summary of data on basal metabolism of seven women studied in the calorimeter of the Russell Sage Institute of Pathology

SUBJECT	SYMBOL	AGE	HEIGHT	WEIGHT	AVERAGE LEVEL OF METABOLISM AT TEMPERATURES BELOW 27°C.	TEMP. OF LOWEST METABOLISM	AVERAGE LOWEST METABOLISM	% DECREASE
			cm.	*kg.*	*cal./M²/hr.*	*°C.*	*cal./M²/hr.*	
No. 1	●	35	175	64	35.6	30.5	30.6	14
No. 2	○	25	162	60	35.8	32	28.2	21
No. 3	×	25	165	55	33.1	32	32.8	1
No. 4	◒	21	162	77	33.6	30.5	29.0	14
No. 5	⊙	23	168	56	35.0	28	32.0	9
No. 6	△	42	169	53	36.2	32.4	33.0	9
No. 7	+	26	165	54	36.0	32	31.0	14
				Averages	34.9		30.9	12

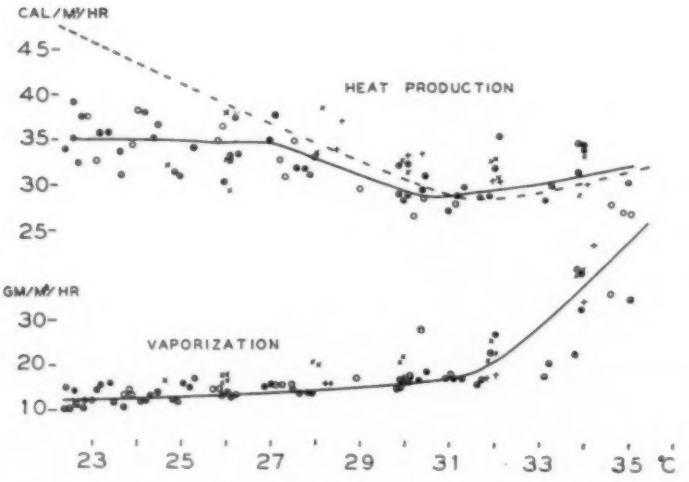

Fig. 1 Upper curve, average basal metabolic rate plotted as a function of calorimeter temperature. Dashed line, average heat loss. Lower curve, average loss of moisture from skin and lungs as affected by calorimeter temperature.

values for the individual subjects. It may be coincidence that the only subject who failed to show at least a 9% fall in metabolism was the one who was receiving hormone therapy. Further study would be needed to establish any such relationship between change in basal metabolic rate and hormone activity.

The change in basal metabolism with environmental temperature observed with these women subjects is the first indication of a "chemical regulation" of body temperature we have been able to find in our studies covering two men and seven women. The level of basal metabolism used for clinical purposes is 36.5 to 37.0 cal./hr./M^2 for women 20 to 35 years of age. In the cold zone, our group, on this basis, has a basal metabolic rate of —5%. Common biologic practice is that of using the metabolic rate in the zone of "thermic neutrality" as the standard of reference. This usage would place our group 19% below the accepted standards. Possibly some allowance should be made in clinical practice for the metabolic adjustment of women to environmental temperature.

2. Water loss from vaporization. In figure 1 are plotted the data for vaporization from skin and lungs. Below 32°C. there was no sweating (except possibly in the axillae) and the water loss was therefore by insensible perspiration. There was a gradual decrease in the water loss as the temperature was lowered from 32°C. to 22°C. This may be due to a drying out of the superficial tissues accompanying the more or less complete vasoconstriction at these temperatures. Above 32°C. sensible sweating was present in nearly all subjects although the amount of sweating was dependent in great part on the amount of activity of the subjects.

3. Rectal temperature. The rectal temperatures of the subjects as affected by environmental temperature are shown in figure 2. The values were higher on the average in the warmer zone than in the cold zone, although the rectal temperatures appeared lowest at 31°C. calorimeter temperature, the point of lowest metabolism. There was a spread of more than 0.6°C.

in the values throughout the whole temperature range, possibly the result of the oestrous cycle or other factor.

4. Average skin temperature. In environments colder than 33°C. the average skin temperature fell almost linearly with surrounding temperature. Above 33°C. the highest observed

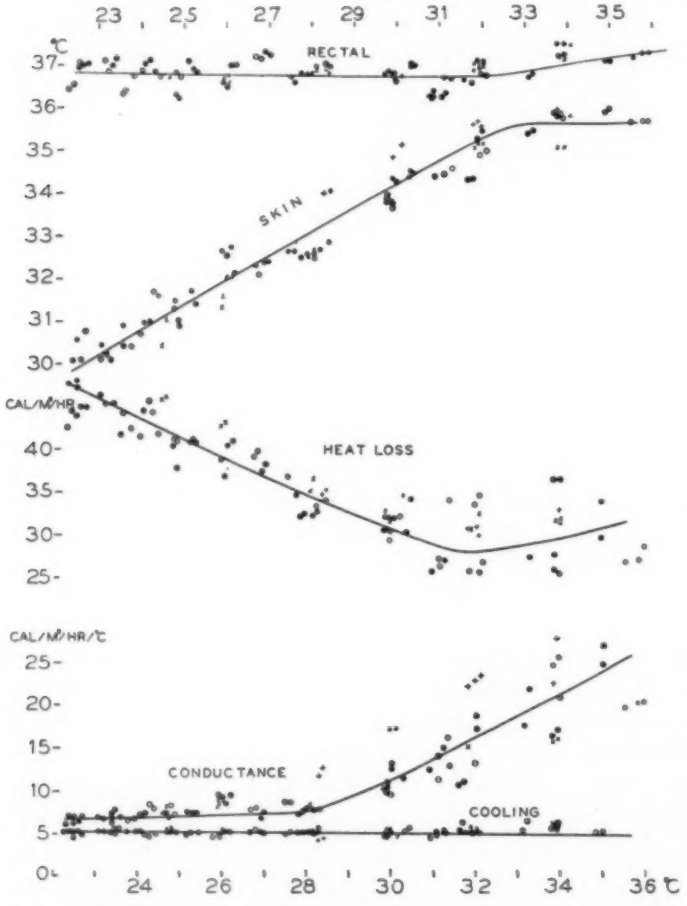

Fig. 2 Relation of calorimeter temperature to rectal temperature, skin temperature, heat loss, peripheral conductance, and rate of cooling from the skin.

values of average skin temperature were about 36°C., and the spread in the values was about 1.2°C., or about twice that for the rectal temperature.

The change in average surface temperature was +0.57°C. per degree change in calorimeter temperature. This is nearly 0.1°C. per degree greater than that observed for our male subjects under like conditions. The fact that some of the subjects had more or less constant skin temperature in the hot experiments while others continued to rise is the counterpart of the variable findings in regard to sweating. Winslow, Herrington and Gagge ('38) have pointed out that in the physiological range of environmental conditions, the body vaporizes exactly the amount of water required to eliminate the heat. Thus, sweating and surface temperature responses can be looked upon as interdependent factors in this zone.

5. Heat loss. Figure 2 shows the values of heat loss for all subjects plotted through the experimental temperature range. The responses to temperature for the subjects show some individual characteristics and the curve represents the average response. This average has a low value of 28.5 cal./hr./M^2 at 32°C. and increases to either side. In the colder zone the heat loss increases at the rate of 2.3 cal./hr./M^2 for each degree drop in calorimeter temperature. Above 32°C. there is a considerable spreading of the heat loss values although there is a definite tendency to increase with temperature. This spreading would be expected on the basis that the subjects did not all have the same heat production at the higher temperature; for each subject the heat loss and heat production were nearly the same in the warm experiments. This spread represents a real difference in the response of individual subjects to warm atmospheric conditions.

It is felt that the low values of heat loss at temperatures of 30°C. to 33°C. lend significance to the low values found for heat production. That is, the body was in thermal equilibrium during these changes, and decreased heat loss, caused by higher environmental temperature, was met by decreased metabolism.

Aside from the evident variations in individual response in the hot zone it is of interest to note that in the cold zone the heat loss level for the thin subject was above the average, whereas that for the stout subject was low. As a result of less peripheral tissue, no. 3 had higher conductance than no. 4 and higher rate of vaporization; these two factors account for the differences in their heat loss.

6. Peripheral conductance. The thermal conductance of the superficial tissues can be calculated from the heat loss and the skin and rectal temperatures. Its importance as an index of effective vasomotor action in the periphery has been discussed (Hardy and Soderstrom, '38). Below 28°C. the conductance decreases slowly as the temperature falls. In this temperature range the level and rate of change of conductance were approximately the same for all subjects. Above 28°C. the conductance rose rapidly indicating superficial dilatation. In this temperature range two rates of increase were observed; the lower rate (the same as observed in the men subjects) was 1.7 cal./M²/hr./°C. per degree rise in calorimeter temperature; the higher rate was 3.3 cal./M²/hr./°C. per degree. Number 7 who complained of difficulty in warm atmospheres showed an abnormally high conductance. The conductance was so high that the vessels became engorged and an edema resulted. This was her complaint in summer weather.

It is generally accepted that the change in conductance in warm atmospheres is brought about by control of blood flow in the superficial tissues. Hardy and Soderstrom ('38) point out that the conductance in the cold zone is constant for the men studied in the calorimeter. With the women the conductance decreased 1.56 cal./M²/hr./°C. when the calorimeter temperature changed from 28°C. to 22°C. This is a significant change and may be due to a slow vasoconstriction of deeper vessels or to the gradual cooling of the body. In any case, this change in conductance represents a deeper penetration of the thermal gradient into the body. Thus, at 22°C. the average depth of the gradient is estimated at 24 mm. and at 28°C. the depth is 19 mm. Thus, below 28°C. one may have a vasomotor

effect extending to the deeper vessels whereas in the warmer atmospheres the activity is in the superficial vessels. A most important adjustment to environmental temperature change in the warm zone is accomplished by the changes in blood flow in the peripheral vessels, but little protection against cold is offered by the conductance change below 28°C.

No explanation is at hand to account for the two separate responses above 28°C. It is possible that this is an individual reaction depending upon acclimatization, etc. The one fact which is common to all subjects is that within our experimental temperature range, the conductance above 28°C. increases in direct proportion to the environmental temperature.

7. Newton's law of cooling. A check on the overall calorimetric and thermometric techniques is the value of K in Newton's cooling law. The value for the women subjects was 5.3 ± 0.4, within the experimental range. The largest deviations were at the higher temperatures as would be expected. This was the same value as found for the men and means only that the thermal heat loss per degree (the total heat loss minus the loss due to vaporization) was the same for both sexes. This was to be expected as the general configurations of the subjects were the same.

8. The average body temperature. Much discussion has centered around the proper formula for measuring the heat lost or stored in the body by means of changes in rectal temperature and average skin temperature. Burton ('35) proposed the formula $0.67\ R + 0.33\ S$ (R = rectal temperature; S = average skin temperature). Hardy and Du Bois found the formula $0.8\ R + 0.2\ S$ best fitted their observations on men. Winslow, Herrington and Gagge found Burton's formula more valid for their observations for colder environments. In order to test the matter further, the fifty observations on the women subjects during basal periods have been analyzed. In figure 3 are plotted all the data for observed and estimated changes in body temperature. The observed change in body temperature was computed using 0.83 as the average specific heat of the body.

In the upper left of figure 3 are plotted the differences in the estimated and observed changes in body temperature as a function of the calorimeter temperature; that is, the estimated temperature change minus the observed temperature change is plotted as ordinate and calorimeter temperatures as abscissae. The spread of the values is shown in the small

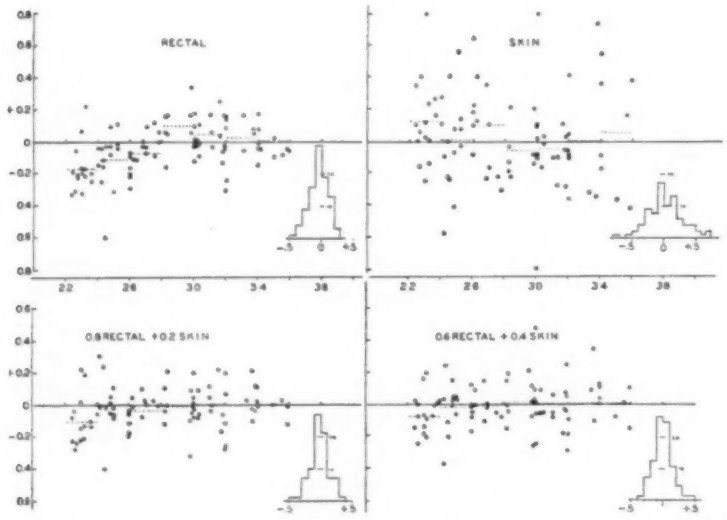

Fig. 3 Graphic analysis of change in average body temperature estimated in four ways as affected by calorimeter temperature. Abscissae calorimeter temperature; ordinates difference in the actual change in body temperature and the estimated change; each point represents change during an hour. Small histograms represent spread of estimated values about the true value. Abscissae degrees Centigrade. Ordinates number of measurements.

histograms. It is evident that in the colder atmospheres the rectal temperature change failed to give a true index of body temperature change, as the body cooled much more than the rectal temperature showed. This difference was less as the calorimeter temperature increased, and above 30°C. the change in rectal temperature on the average was a good index of body temperature change. Of the ninety-four measurements shown,

seventy-seven of them gave the true change in body temperature within $\pm 0.2°C.$

The upper right of the figure shows the skin temperature as an index of body temperature. At low temperatures the skin cooled more than the body, and above 28°C. the skin temperature served as a fair index of body change. The spread of the values of body temperature calculated from skin temperature was large, only forty-eight of the eighty-six readings falling within $\pm 0.2°C.$ of the actual change.

That the skin temperature overshoots the mark and the rectal temperature undershoots it lends confidence to the hope that a combination of the two will give a fairly accurate estimate of the body temperature change. The lower left shows the estimation by the formula suggested by Hardy and Du Bois and the lower right the estimation approximating that of Burton. The temperature range from 22°C. to 24°C. shows both formulae to fail, the Hardy and Du Bois formula by $-0.10°C.$ and the Burton formula by $-0.06°C.$ From 26°C. on up either formula works, with slightly better estimates obtained by the Burton method. It is evident that a much greater weight than 40% must be given to the skin when temperatures below 24°C. are reached. It is not surprising that no single formula can be used for the whole temperature range or for all subjects. The women, having more subcutaneous fat would be expected to differ slightly from men toward greater weighting of the skin temperature. Above 24°C. good estimates of change in body temperature may be obtained with either 80% R + 20% S or 60% R + 40% S. Below 24°C. where heat is being lost very rapidly, a formula of 50% R + 50% S may give better estimates. This means, however, that very careful measurements of average skin temperature must be made at these lower temperatures if good estimates of change in average body temperature are to be made.

9. Radiation, convection, vaporization. The partition of the total heat loss at different temperatures is shown in figure 4. Vaporization loss at 22°C. amounts to only 15% of the total

and it is probable that more than half of this is from the lungs. At 32°C. just before the onset of sweating the vaporization loss was 40% of the total. This increase in importance of vaporization is the result of decreasing heat loss rather than increase in actual amount of vaporization. Radiation at 22°C. is greater in magnitude than the heat production and amounts to more than 70% of the total heat loss. Between 35°C. and

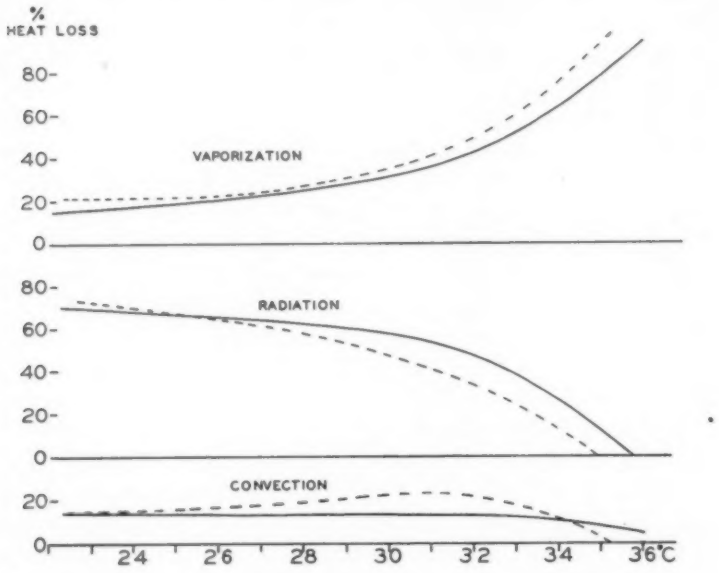

Fig. 4 Average partitions of heat loss at different calorimeter temperatures of men and women. Abscissae, calorimeter temperature; ordinates, per cent of total heat loss. Solid line, women; dashed line, men.

36°C. radiation becomes zero, this being the point at which the average skin temperature is the same as the calorimeter temperature. The radiation loss in per cent is maximal under the conditions of these experiments, the subjects lying nude and motionless. With activity the actual amount of radiation will increase in the cold atmospheres and decrease in the warm atmospheres. The per cent of heat lost by convection increases

slightly with lower calorimeter temperatures but ranges between 5% and 15% throughout the experimental range. Convection loss falsely indicates a positive value even when the skin temperature is below that of the calorimeter. This artifact is probably due to the technical difficulty of operating the calorimeter at such high temperatures. Values of heat loss measured in this range may be in error by 5 or 6 calories per hour, an amount sufficient to account for the anomalous results shown in the chart.

10. Comparison of the responses of nude and motionless male and female subjects to different calorimeter temperatures. In one respect there was no demonstrable sex difference in the responses to environmental temperature, namely, the rate at which the body cools per degree difference in skin and calorimeter temperature. Aside from this factor, however, there were significant differences in all of the quantities studied. Figure 5 shows the comparison of average values.

The rectal temperature of the men decreased very uniformly with environmental temperature, whereas that of the women showed a low point at about 31°C. On either side of this environmental temperature the rectal temperature of the women was higher and in some of the cool experiments the women had distinctly higher internal temperatures.

In the environmental temperature range below 30°C. the average surface temperature of the women changed 0.57°C. for a change of 1 degree in calorimeter temperature. The average surface temperature of the men changed 0.48°C. on the same basis. Thus, in the cool zone the surface temperature and the average body temperature of the women were lower than that of the men exposed under the same conditions. In the warmer atmospheres the surface temperature of the women rose to a higher level than that of the men (1 to 1½ degrees). Thus, their average body temperature was warmer under these conditions than that of the men. This difference in the change of surface temperature with environmental temperature accounts for the differences in per cent heat lost by radiation, shown in figure 4, in the cold and warm atmospheres. This

difference is much more pronounced at the higher temperatures in which the women radiate much more of their heat than do the men.

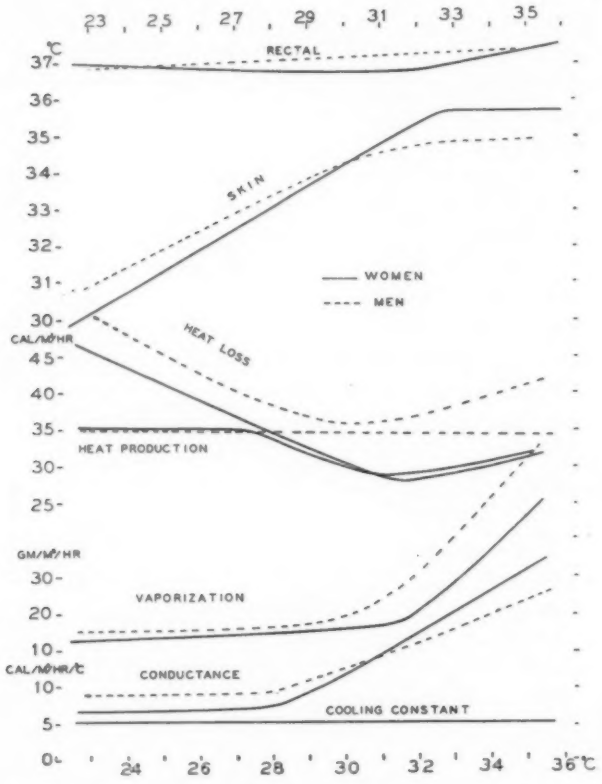

Fig. 5 Comparison of average values of various factors for women and men at different calorimeter temperatures.

The heat loss of the women is on an average 3 to 4 cal./hr./M² less than that of the men in the temperature range below 28°C. The rate of increase of heat loss as the calorimeter temperature is lowered is approximately the same for both sexes, that is, the heat loss increases 2.2 cal./hr./sq.M for each degree

fall in calorimeter temperature for the women and 2.4 cal./degree for the men. The striking difference in the heat loss curves is seen in the low values reached by the women. Values as low as 25 cal./hr./M² have been found for the women, whereas 36 cal. is the low point for men. The low point of heat loss for the men was found at calorimeter temperature 30°C. and for the women at 31.5°C.

The basal heat production of the women and the men was the same and level at 35 cal./hr./M² for calorimeter temperatures lower than 27°C. In both sexes no change in metabolism was observed in the colder range until the actual onset of a chill, and no "chemical regulation" as a response to low temperature was observed in this range. Above 28°C. the heat loss for women was the same as the heat production and both decreased with higher temperature to the low point at 30°C. to 32°C. No such phenomenon was observed for the men whose basal heat production was constant throughout the experimental range. That the change in the basal heat production which was observed in women was a real response to environmental temperature is supported by the corresponding changes observed in the heat loss for the women and not for the men. The range of vasomotor regulation of the women is seen to be larger than that of the men as the latter have only the 2-degree range from 29°C. to 31°C., whereas the women appear to have the 5-degree range from 27°C. to 32°C.

Vaporization for the men was higher in the cold zone than for the women. This may be due to the fact that women can more completely cut off the peripheral circulation and a dryer skin surface is a consequence of slower transudation in women. The men began to sweat visibly at about 30°C. whereas the women, because of their lowered heat production, did not need to sweat until the temperature was between 32°C. and 33°C. The amount of sweating in the women was progressively less than in the men, as the temperature was raised. The per cent heat lost by sweating for the women was also less than for the men at all temperatures except between 27°C. and 29°C. It has been pointed out that a definite relationship might be

expected between the basal heat production in men and the water vaporized as long as the temperature did not rise above 29°C. For the women the temperature would have to be lower than 27°C. and even there the relationship would be unsatisfactory as the water loss decreased with lower temperature.

The thermal conductance of the superficial tissue would be expected to be higher for the men than the women from a consideration of the levels of heat loss for the sexes. The conductance for the women was estimated as 2.2 cal./hr./M²/°C. lower than for the men, at 23°C. On the basis of the measured specific thermal conductivity of fat and muscle tissue, this corresponds to about 5 mm. of extra superficial tissue for the women. The conductances of both sexes increased slightly as the calorimeter temperature was raised. Between 28°C. and 29°C. both sexes showed definite vasomotor response in the superficial vessels. Three of the women subjects had the same degree of response as the men. Three of the women, however, increased their blood flow at twice that rate. Both the level of conductance and the rate of increase may represent individual responses to temperature in these warm atmospheres. Racial differences, acclimatization, and other factors should be important. So far as conductance is concerned, the only real difference between the sexes was found in the lower temperature range.

SUMMARY

Eight normal women have been studied in the calorimeter of the Russell Sage Institute of Pathology in regard to the mechanism of heat loss and heat production at environmental temperatures ranging from 22° to 36°C. The women were lying nude under basal conditions on a comfortable bed within the calorimeter. The results have been compared with those obtained on two men, using exactly similar procedure. The basal metabolic rate of the women in the colder zone was about 35 cal./sq.m./hr. and approximately the same as that of the men. In the warmer zone the women showed a marked drop in basal metabolism until it averaged 30.9 cal./sq.m./hr. at

31°C. The men showed no such drop. This indicates a type of regulation of body temperature which was not found in the men whose metabolism had remained level. In the women the fall in basal metabolism was concomitant with marked decrease in heat loss in the comfort zone, reaching at 31.5°C. as low a level as 28.5 cal./sq.m./hr. The lowest point observed for the men was 34.9 cal./sq.m./hr. In the warmer zones there was considerable variation in the individual response.

The average skin temperature for the women in the hot zone was higher than that for the men by 1.5°C. and lower in the cold zone by 1.0°C. The conductance of the peripheral tissues for the women was 20% lower than that of the men in the cold experiments, representing 20% more insulation against cold. Sweating was less marked in the women and did not begin until the environmental temperatures were higher than was the case for the men. In general, there were slight differences in every single factor of temperature regulation, and in all respects the women had physiological advantage, but especially in the reduction in heat loss and heat production in the warmer comfort zone.

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A QUANTITATIVE STUDY OF VITAMINS IN THE RUMEN CONTENT OF SHEEP AND COWS FED VITAMIN-LOW DIETS

IV. PANTOTHENIC ACID

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(Received for publication November 8, 1940)

In previous communications (McElroy and Goss, '40 a, '40 b, '40 c; McElroy and Jukes, '40) we have reported that riboflavin, vitamin K, pyridoxine, thiamine and the anti egg-white-injury factor were found in the rumen contents of animals fed a ration deficient in these vitamins. In the present investigation evidence was found for the formation of pantothenic acid in the rumen of the experimental sheep and cows.

EXPERIMENTAL

The care and treatment of the experimental ruminants has been described in an earlier paper (McElroy and Goss, '40 a).

Pantothenic acid assays. The "filtrate factor" method of Jukes ('37) was used in estimating pantothenic acid. Chicks were placed on an ordinary mixed diet for 6 to 8 days after hatching and then depleted for 8 to 10 days on heat-treated diet 80G composed of natural foodstuffs. The chicks were then weighed and divided into groups. Each series was controlled by a group on the basal diet made up of 100 gm. of the heated diet 80G + 4 cc. of a rice bran filtrate which had previously been shown to produce a maximal growth response when fed at a level of 3.5 cc. per 100 gm. of diet. When maximal growth response was produced under the conditions of the assay the diet was said to contain at least 1 filtrate factor unit per gram.

Jukes ('39) has shown that 1 filtrate factor unit is equivalent to 14 μg. of pantothenic acid.

The results of an assay of dried sheep rumen and reticulum contents and of the ruminant ration are summarized in table 1. The reader is referred to the original paper of Jukes ('37) for details of the method of calculating results. The pantothenic acid activity of the rumen and reticulum contents was estimated to be 5 chick units (70 μg.) per gram as compared with less than 0.2 chick units (2.8 μg.) per gram for the ruminant ration.

TABLE 1

Pantothenic acid assay of ruminant ration and of dried sheep rumen and reticulum contents. Supplements were added to diet 80G. Ten chicks were included in each group

SUPPLEMENT IN 100 GM. OF DIET	AVERAGE GAIN IN WEIGHT IN 8 DAYS	NUMBER OF CASES OF SPECIFIC DERMATITIS AFTER 8 DAYS
	gm.	
1. None	1.7	9
2. 15 gm. ruminant ration	— 6.0	5
3. 15 gm. rumen and reticulum contents	29.2	0
4. 4 cc. rice bran filtrate (+ control)	38.4	1 (mild)

SAMPLE OF CALCULATIONS:

$$\text{Pantothenic acid per gram} = \frac{29.2 - 1.7}{(38.4 - 1.7)\ 0.15} = 5 \text{ chick units or } 70\ \mu g.$$

The same deficient ration which was fed to sheep was also fed to cows. One of the experimental animals, no. 557, had a fistula into the rumen as described in an earlier paper (McElroy and Goss, '40 a). Samples of rumen contents from two of these animals and milk from one of them were assayed for pantothenic acid. Typical results are summarized in tables 2 to 4. From table 2 it was estimated that the sample of rumen contents obtained from the non-fistulated cow no. 706 contained 61 μg. of pantothenic acid per gram dry matter. This sample contained a large amount of sand and grit (McElroy and Goss, '40 c) and consequently it is believed that this is an excessively low estimate of the true value during the experimental period. More representative samples of rumen contents were obtained from the fistulated cow no. 557 and a

typical assay of a composite sample of this material is given in table 3. The pantothenic acid content of this sample was estimated to be 94 μg. per gram.

Table 4 shows that dried skim milk prepared from the milk of cow no. 557 contained 46 μg. of pantothenic acid per gram.

TABLE 2

Pantothenic acid assay of dried rumen contents from non-fistulated cow no. 706. Supplements added to heated diet 80G

SUPPLEMENT IN 100 GM. OF DIET	NUMBER OF BIRDS	AVERAGE GAIN IN WEIGHT IN 11 DAYS	NUMBER OF CASES OF SPECIFIC DERMATITIS AFTER 11 DAYS
		gm.	
1. None	3	—1.3	3
2. 12 gm. dried rumen contents	6	32.4	2
3. 4 cc. rice bran filtrate	8	62.7	2 (mild)

Estimated pantothenic acid, 61 μg. per gram.

TABLE 3

Pantothenic acid assay of dried rumen contents of fistulated cow no. 557. Supplements added to heated diet 80G.

SUPPLEMENT IN 100 GM. OF DIET	NUMBER OF BIRDS	AVERAGE GAIN IN WEIGHT IN 10 DAYS	NUMBER OF CASES OF SPECIFIC DERMATITIS AFTER 10 DAYS
		gm.	
1. None	5	5.8	5
2. 8 gm. dried rumen content	7	34.0	2
3. 6 cc. rice bran filtrate	7	58.0	0

Estimated pantothenic acid, 94 μg. per gram.

TABLE 4

Pantothenic acid assay of dried milk, fistula cow no. 557. Supplements added to heated diet 80G.

SUPPLEMENT IN 100 GM. OF DIET	NUMBER OF BIRDS	AVERAGE GAIN IN WEIGHT IN 12 DAYS	NUMBER OF CASES OF SPECIFIC DERMATITIS AFTER 12 DAYS
		gm.	
1. None	6	10.9	6
2. 20 gm. dried skim milk [1]	7	40.8	0
3. 4 cc. rice bran filtrate	7	55.7	0

Estimated pantothenic acid, 46 μg. per gram.

[1] The skim milk used was prepared from milk collected between the seventeenth and twenty-fourth and between the forty-fifth and forty-ninth days of lactation. Equal weights of the two preparations were combined to make the 20% supplement.

During the period when this milk was being collected this cow was giving 20 pounds of milk per day. Assuming that about 10 pounds of milk are required to produce 1 pound of dry skim milk, then the daily excretion of pantothenic acid by way of the milk would be approximately 41 mg. The maximum food consumption of the cow during this period was 6 kg. per day of a ration containing less than 2.8 mg. of pantothenic acid per kilogram, or a total of about 17 mg. per day as compared with a daily excretion in the milk of 41 mg.

In an earlier paper (McElroy and Goss, '40 b) we discussed the question of differential passage of materials from the rumen as a possible cause of the increase in the vitamin potency of rumen contents. It does not seem to appear possible that a twenty- to thirtyfold increase in pantothenic acid concentration of the rumen contents over that of the ration fed could be explained in this manner. Our results with milk and with rumen contents indicate that appreciable quantities of pantothenic acid may be formed in the rumen, probably by bacterial growth.

SUMMARY

1. Sheep and cows were fed a vitamin B complex low diet containing less than 2.8 μg. of pantothenic acid per gram. The rumen and reticulum contents of the sheep were found to contain 70 μg. of pantothenic acid per gram dry substance or a twenty-fivefold increase over the ration fed.

2. Samples of rumen contents from two cows fed the deficient ration contained from twenty to thirty times as much pantothenic acid as did the ration fed.

3. The amount of pantothenic acid excreted by way of the milk from one of the cows was estimated to be twice as great as could be accounted for in the ration fed.

ACKNOWLEDGMENT

We wish to express our appreciation to Dr. T. H. Jukes for provision of facilities in his laboratory for the conducting of the pantothenic acid assays and for his many helpful suggestions.

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URINARY EXCRETION OF THIAMINE ON HIGH FAT AND HIGH CARBOHYDRATE DIETS[1, 2]

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ONE FIGURE

(Received for publication October 17, 1940)

As early as 1914 Funk made the observation that pigeons on a diet deficient in thiamine and high in carbohydrate developed polyneuritis earlier than pigeons on a diet high in fat but similar in other respects. Since that time a number of research workers have investigated further the relationship between the thiamine requirement of animals and the percentage of carbohydrate in the diet. Evans and Lepkovsky ('29), for instance, reported that young rats required less thiamine for growth on thiamine-low diets when some of the carbohydrate of the diet was replaced by fat. In 1937 Salmon and Goodman reexamined the question and their results confirmed earlier reports on the "thiamine-sparing" action of fat. More recently Stirn, Arnold and Elvehjem ('39) after a careful experimental study of the problem report that when carbohydrate in a thiamine-deficient ration is isocalorically replaced by fat the resultant diet will ameliorate the condition of polyneuritic rats. It is interesting to note that experiments

[1] Presented before the Section of Biological Chemistry at the One Hundredth Meeting of the American Chemical Society at Detroit, Michigan, September 9–13, 1940.

[2] We wish to express our thanks to Dr. Richard M. Johnson, recent Medical Director of the Wm. J. Seymour Hospital at Eloise, Michigan, and at present Medical Director of Frederick Stearns and Company, Detroit, for providing us with some of the materials needed in this investigation.

with dogs indicate that these animals also require more thiamine on a high carbohydrate than on a high fat regimen (Arnold and Elvehjem, '39).

The purpose of the present investigation was to determine whether or not alterations in the ratio of fat to carbohydrate in the diet of human adults are reflected in the urinary thiamine output when the dietary intake of thiamine remains constant. The urinary thiamine excretion has been found to be a useful index of thiamine deficiency (compare Robinson, Melnick and Field, '40), and it is important that all factors which might possibly influence the thiamine excretion be studied. The excretion of thiamine in the urine is a sensitive index of the vitamin intake, a change from a diet adequate in thiamine to one inadequate in this vitamin being promptly reflected by a decrease in the urinary output of vitamin B_1 (compare Melnick, Field and Robinson, '39). It was, therefore, thought possible that if a high carbohydrate diet resulted in an increased demand for thiamine by the body this fact might be indicated by a change in the urinary output of thiamine within a relatively short period.

There are conflicting reports in the literature regarding the effect of a high carbohydrate diet on the urinary thiamine excretion. Schroeder ('39) reported that large doses of sugar given daily to two patients on a normal diet eventually caused the urinary thiamine output to become nil. On the other hand, in a report which appeared after the present investigation was begun Wang and Yudkin ('40) conclude that changes in the carbohydrate content of the diet affect the urinary thiamine output very little, if at all.

EXPERIMENTAL

In the present investigation the urinary thiamine excretion of individuals on comparable high carbohydrate and high fat diets was studied. The subjects were all males from 20 to 30 years of age. In one series of experiments, on each of 3 consecutive days individuals were given a loaf, or a definite fraction of a loaf, of bread and 1000 calories of sucrose.

In addition, 4 gm. of brewers' yeast were eaten by the subjects daily. On the third day a 24-hour sample of urine was collected for determination of its thiamine content. During a similar 3-day period, the same individuals were given a diet identical in all respects except that the sucrose was replaced isocalorically with butter. The amount of bread eaten each day by any one individual was exactly the same during the two 3-day periods. On the third day the urine was collected as before and the thiamine in the sample was determined chemically essentially according to the method of Melnick and Field ('39). The 24-hour urine samples of any one individual—i.e., the urine collected during a period when the diet was high in fat and when it was high in carbohydrate—were analyzed for thiamine on the same day to make conditions as uniform as possible. Melnick and Field ('39) have shown that when the urine is preserved with acid and toluol no loss of thiamine occurs for a considerable period of time.

The results of the experiment are shown in the first part of table 1. Although the urinary thiamine output varies from individual to individual, it was found that an increase in fat at the expense of carbohydrate in the diet of any one individual did not significantly alter the urinary thiamine excretion. Changes in the ratio of the calories of fat to the calories of carbohydrate in the diet varying as widely as 1:0.75 to 1:50 did not significantly influence the urinary excretion of thiamine in these experiments.

In a comparable study one individual (W.M.C.) was given an experimental diet for 12 days. For alternate 3-day periods the diet was first high in carbohydrate and then in fat. The diet consisted of "whole" wheat bread, 4 gm. of dried yeast, and either 1000 calories of fat or of carbohydrate, depending upon the period. Here again radical changes in the ratio of fat to carbohydrate in the diet did not change significantly the output of thiamine in the urine. The only noteworthy alteration was a moderate increase in the excretion of thiamine as the experiment progressed.

It was thought that perhaps in the foregoing experiments the high carbohydrate diets did not depress the thiamine output because the vitamin B_1 content of the diets was too high. In a second series of experiments, therefore, individuals were given thiamine-low diets consisting of white bread and butter,

TABLE 1

Urinary excretion of thiamine by normal adults on comparable high fat and high carbohydrate diets

SUBJECT	WEIGHT	DIETARY THIAMINE INTAKE	DAILY DIET					RATIO OF FAT TO CARBOHYDRATE IN DIET	THIAMINE EXCRETED ON 3RD DAY
			Yeast [1]	Bread [2]	Butter	Sucrose	Total		
	kg.	*μg./24 hrs.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.*	*Cal.F:Cal.C*	*μg./24 hrs.*
C.A.K.	78.5	1420	14	1303	1000	....	2317	1: 1.08	284
		1420	14	1303	....	1000	2317	1:43.2	304
E.A.H.	67	1480	14	1418	1000	....	2432	1: 1.09	229
		1480	14	1418	....	1000	2432	1:41.6	222
H.B.	68.5	1290	14	1073	1000	....	2087	1: 0.84	256
		1290	14	1073	....	1000	2087	1:47.7	264
M.F.P.	63	1480	14	1418	1000	....	2432	1: 1.09	193
		1480	14	1418	....	1000	2432	1:41.6	174
W.W.	79	1210	14	957	1000	....	1971	1: 0.75	156
		1210	14	957	....	1000	1971	1:50.8	168
W.M.C.	68	1420	14	1303	1000	....	2317	1: 1.08	270
		1420	14	1303	....	1000	2317	1:43.2	298
E.R.J.	65.5	155	..	1371 [3]	1000	....	2371	1: 1.04	87
		155	..	1371	....	1000	2371	1:34.6	89
H.W.	70	170	..	1492 [3]	1000	....	2492	1: 1.13	78
		170	..	1492	....	1000	2492	1:33.2	71
R.L.	69	170	..	1492 [3]	1000	....	2492	1: 1.13	71
		170	..	1492	....	1000	2492	1:33.2	78
L.K.L.	72	170	..	1492 [3]	1000	....	2492	1: 1.13	66
		170	..	1492	....	1000	2492	1:33.2	66

[1] Brewers' yeast (4 gm.). This yeast contained 168 μg of thiamine per gram. We are indebted to Dr. Daniel Melnick of the University of Michigan for determining the total thiamine content of the yeast.

[2] Unless otherwise noted all the bread used in this study was a "whole" wheat bread obtained from a small bakery. The flour used in its preparation consisted of three-quarters whole wheat and one-quarter white flour. The fat and carbohydrate content of the bread was calculated from tables compiled by Sherman ('38) on the basis that the bread was three-quarters whole wheat and one-quarter white. The thiamine content of the bread was also calculated on this basis from tables in Hawk and Bergeim ('37).

[3] White bread.

or of white bread and sugar. Yeast was omitted from these diets. The experimental procedure was the same as in the first series of experiments. The results of this study are also shown in table 1.

The thiamine intake of the second group of individuals studied was much less than that of the first group. This fact is well reflected in the much lower output of thiamine in the urine of those individuals receiving an inadequate amount of thiamine in the diet. Here again, however, it was found that radical changes in the ratio of fat to carbohydrate in the diet were not reflected by any significant variation in the urinary thiamine output.

In a study bearing on the diagnosis of thiamine deficiency Robinson, Melnick and Field ('40) have shown that the percentage of an oral dose of 5000 μg. of thiamine which is excreted in the urine is lower in the case of vitamin B_1-deficient individuals than in normal subjects. Thiamine-deficient individuals excrete less than 7% of the test dose. Advantage was taken of this test in another experiment designed to study the effect of carbohydrate on the urinary thiamine output. An individual (W.M.C.) was given a thiamine-low diet consisting of white bread and 1000 calories of butter daily. The urinary thiamine was determined on alternate days. On the ninth day of the experiment 5000 μg. of thiamine were taken orally by the subject and the urinary thiamine output of the following 24-hour period was similarly determined. After an interval of 2 weeks, during which time the subject consumed a normal diet, the experiment was repeated with the fat in the diet replaced isocalorically by sucrose. The amount of bread eaten daily during the two periods was the same. The results of the study are shown in figure 1.

This latter study yielded results comparable to those of the other experiments reported in this paper. Whether the low thiamine diet was high in fat or high in carbohydrate, the picture of urinary thiamine excretion was essentially the same. In both cases the excretion of thiamine dropped rapidly and reached a similar level. In the case of the experiment with

a high fat diet, 5.9% of the oral dose was excreted in the urine, while in the case of the study with a high carbohydrate diet 5.6% of the oral dose was excreted.

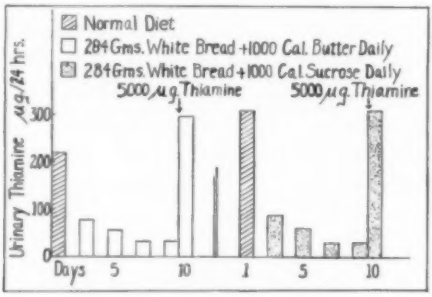

Fig. 1 Urinary excretion of thiamine administered orally after a period of depletion on comparable high fat and high carbohydrate diets.

DISCUSSION

The choice of the rather simple experimental diets was dictated by the desire to have as few variables as possible, as well as by the necessity of having easily reproducible diets of known composition which could be eaten by the cooperating subjects with a minimum of effort on their part, and which would also entail minimum interference with their time schedule. While the diets were not altogether adequate in all the known essential food factors, the rather short experimental period would seem to excuse this. On the other hand, it was thought that if any significant alterations of the urinary thiamine excretion were effected by great distortions of the ratio of fat to carbohydrate in the diet they would make themselves apparent within the experimental period. This seems probable in view of the fact that alterations in the amount of thiamine ingested reflect themselves almost immediately by changes in the urinary thiamine output as clearly shown in figure 1.

It has been said that "fat spares thiamine," and while it seems established that under certain conditions the thiamine requirement of growing animals is influenced by the percent-

age of fat in the diet, it is interesting to note that analyses of the tissues of rats, chickens, and pigs fed on rations deficient in thiamine and high and low in fat, respectively, have indicated that the thiamine content of the tissues is dependent upon the thiamine content of the ration and not upon the fat content (Kemmerer and Steenbock, '33). The amount of fat or carbohydrate in diets of like thiamine content did not differentially have any influence on the thiamine content of the tissues. The observations reported in the present study are somewhat comparable. The finding in the present investigation that, under the conditions described, alterations of the fat-carbohydrate ratio of the diet have no significant effect upon the urinary elimination of thiamine of adult human subjects does not, of course, gainsay the conceivable significance of this ratio in nutrition, particularly over long periods and with growth as part of the picture.

The present results indicate that in tests involving a study of the urinary thiamine excretion of patients, no concern need be given to the fat-carbohydrate ratio of the diet eaten just prior to the determination. This fact is of practical importance, since tests of this nature are used as aids in the diagnosis of thiamine deficiency (compare Robinson, Melnick and Field, '40).

SUMMARY

In an attempt to determine whether the carbohydrate content of the diet, like the thiamine content, influences the urinary output of thiamine, a study was made of the urinary thiamine excretion of adult individuals on comparable high fat and high carbohydrate diets. In all of the experiments reported it was found that even great alterations in the ratio of fat to carbohydrate in diets of the same thiamine content did not significantly affect the urinary thiamine excretion. This was true whether the thiamine intake was adequate or fairly low. A study of the excretion of thiamine administered orally after a period of depletion on comparable high fat and high carbohydrate diets also revealed no differential

influence of fat or carbohydrate on the urinary thiamine output. The bearing of these findings on the validity of tests for thiamine deficiency which are based on a determination of the amount of thiamine excreted in the urine is pointed out.

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THE OPTIMUM LEVEL OF PROTEIN INTAKE FOR THE GROWTH AND FATTENING OF SWINE [1]

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(Received for publication October 26, 1940)

INTRODUCTION

The choice of the level of protein when expressed as a percentage of the ration to be fed to pigs, presents a major problem in the nutrition of this species. Contributing factors to the complexity of this problem are: (a) the relatively rapid growth rate of swine, which exceeds that of any other class of livestock, (b) possible decreasing requirements for protein during the progress of growth, and (c) economic considerations, especially those concerning the relatively high price of protein supplements.

Carroll and Mitchell and associates ('32, '34, '35, '36, '37) have investigated by means of growth and nitrogen balance studies the question of the changing requirements of pigs at different stages of growth, and have shown that for pigs weighing less than 100 pounds the ration should contain more than 17% of a protein mixture obtained from corn, tankage, soybean oil meal or linseed oil meal, and alfalfa meal. For pigs weighing 150 pounds, 17% of protein was found to be adequate and 15% was enough for pigs weighing 175 to 200 pounds. For pigs weighing 40 to 100 pounds, protein levels

[1] Presented in part before the Division of Agricultural and Food Chemistry at the Boston Meeting of the American Chemical Society, September, 1939.

Authorized for publication on October 23, 1940, as paper no. 996 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

up to and exceeding 22% were indicated, by nitrogen balance studies, as being necessary for maximum growth.

Recently Carroll and Burroughs ('39) in a preliminary report of dry-lot group-feeding trials have shown that young pigs must receive a higher percentage of protein than is sufficient for heavier pigs, and their data suggest that the ration of 50-pound pigs should contain at least 18 to 20% of protein or perhaps more. Between the weights of 75 and 100 pounds their data do not indicate any advantage in gains for more than 15% of protein in the ration, and 12% of protein is indicated for pigs weighing from 150 to 200 pounds.

Woodman, Evans, Callow and Wishart ('36) have concluded from the results of individual feeding trials and group feeding tests that a feeding treatment providing 12% of fish meal (17.48% crude protein) from weaning to 90 pounds live weight, 10% of fish meal (15.93% crude protein) from 90 to 150 pounds, and 5% of a mixture of equal parts of meat meal and ex. soya-bean meal (12.25% crude protein) in the final period will supply all of the digestible protein for the maximum rate of growth permitted by the net energy content of the ration. Higher amounts of protein in the ration were of no benefit and actually depressed the rate of live weight increase in some cases.

From these reports it would appear that, while the minimum protein levels which will produce good growth in pigs are fairly well recognized, the relative value of higher levels of protein intake is not well established. Experiments dealing with the general problem of how much protein it is nutritionally advisable to feed to growing pigs have been conducted by the authors since 1936 and this report presents some of the results of this research.

EXPERIMENTAL

Four experiments were conducted. The number of pigs used in each experiment, the protein levels compared, and the approximate intervals of body gain studied are given in table 2.

The composition of the rations is given in table 1. Number 2 yellow corn was used throughout the experiments. The tankage was a 36% protein, steam rendered by-product of a local abattoir and was relatively high in bone content. The soybean oil meal used in these experiments was processed by the expeller method from beans of the Illini, Manchu, Dunfield, Richland and Mandell varieties. The alfalfa meal was produced from local sun-dried hay by passing it through a hammer

TABLE 1

Composition of rations

INGREDIENT	EXPERIMENT 1		EXPERIMENT 2		
	PER CENT PROTEIN		PER CENT PROTEIN		
	10	15	15	20	25
	%	%	%	%	%
Yellow corn	93	76	76.5	57.5	42.5
Tankage	1	15	15	20	18
Soybean oil meal	1	4	4	18	35
Alfalfa meal	4	4	4	4	4
Salt	0.5	0.5	0.5	0.5	0.5
Ground limestone	0.5	0.5	....	....	....

INGREDIENT	EXPERIMENT 3			EXPERIMENT 4		
	PER CENT PROTEIN			PER CENT PROTEIN		
	12	17	22	17	22	27
	%	%	%	%	%	%
Yellow corn	87.5	69.5	51.5	65.5	45.5	36.5
Tankage	4	13	22	12	22	33
Soybean oil meal	4	13	22	12	22	20
Alfalfa meal	4	4	4	10	10	10
Salt	0.5	0.5	0.5	0.5	0.5	0.5

mill. One-half of 1% of salt and 1% of ground limestone were included in the rations of experiment 1. The limestone was omitted in experiments 2, 3 and 4, since the chemical analysis indicated that the additional calcium was not necessary and did not influence the calcium-phosphorus ratio to any marked extent. In addition direct sunlight was available in all of the experiments.

The rations were designed to provide the different levels of protein intake indicated in table 1. No determinations were made of the digestibility of the protein or of its biological

value. Rather the scope of these experiments was limited to a study of the relative values of the gross levels of protein intake for pigs of different ages, it being felt that a study of the influence of the level of protein intake and of the stage of development of pigs, on the digestibility and biological value of the rations constituted a separate problem for later study. However, due to the similarity in the constitution of the various rations it is not thought that their digestibilities are markedly different.

Pigs of the Poland China, Chester White, Duroc Jersey and Berkshire breeds were used. They were housed in an experimental feeding house provided with a concrete floor and having outside concrete runways. They were fed as matched pairs in experiment 1 and as matched triplets in the other trials. The experimental pairs or triplets were selected to be of the same breed, sex, age and of similar weight, and members of each pair or triplet were fed the same daily quantity of feed. The amount of feed allotted daily to each member of a pair or triplet was determined by the feed consumption of the pig in each pair or triplet which consumed the smallest quantity of feed; hence, the feed intake was probably restricted to some extent for one member of each pair and two members of each triplet. The pigs were fed three times daily in individual stalls especially constructed for these experiments. They were weighed weekly, and the initial and final weights as given in table 2 are the averages of weights taken on 3 successive days.

RESULTS AND DISCUSSION

Table 2 is a summary of the results. An analysis by variance was made on the data for individuals for the average daily gains and for the feed requirement for 100 pounds of body gain. There are also given the differences between means which are required to show significance at the 5% point of probability.

In experiment 1 the gains for all of the pigs on the 15% level of protein exceeded those of the pigs on the 10% level and were attained with less feed per unit of gain.

The data for experiment 2 covering the growth span from 84 to 200 pounds, also have been broken down to cover other intermediate weight increments. Gains and food efficiency were less for pigs on the 25% protein level in a number of instances. The comparison for the weight interval 127 to 200 pounds was made with only seven pigs at each level of protein intake, there being only seven triplicates which attained the required initial weight of 127 pounds in comparable time intervals. Significant differences were not obtained with respect to gains or feed requirement between the 15 and 20% protein levels, both of which are indicated as better than the 25% level.

Lighter pigs weighing 40 pounds were used in experiment 3. The 12% protein level proved unsatisfactory for these pigs. On this level three pigs failed to gain in a manner satisfactory enough to be included in the comparison, and the feed requirement for the remaining nine pigs was relatively high as was the variation between individuals. Consistent results were obtained favoring the 17% protein level, although on the 22% protein level similar results were obtained in some of the comparisons. The failure of individual pigs to attain the required weights in time intervals to give valid comparisons necessitated making comparisons, in some cases, between unequal numbers of individuals on the respective protein levels. The number of individuals used on any one level is, therefore, some indication of the merit of that particular protein level.

A summary of the results shows that neither an increased rate of body gain nor increased feed efficiency results from a 25% protein level of feeding as compared with either a 15 or 20% level. A 10% protein level was shown to be inferior to a 15% level as judged by body gains and by feed efficiency. Results obtained with 12 and 22% protein levels showed no significant differences favoring either level with respect to growth. Both of these levels, however, seemed to be less satisfactory than the 17% protein level judging by both the average daily gains of the pigs and the efficiency of their food utilization.

TABLE 2

Summary of average weights, body gains and feed requirements of pigs fed rations of different protein content

NUMBER OF PIGS	AMOUNT OF PROTEIN IN RATION	AVERAGE AGE AT BEGINNING	LENGTH OF EXPERIMENTAL PERIOD	INITIAL BODY WEIGHT	MEAN AVERAGE DAILY GAIN	MEAN AVERAGE DAILY RATION	FEED FOR EACH 100 LBS. GAIN
	%	*days*	*days*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Experiment 1 — 130 to 180 lbs.							
10	10	222	39	131	1.08	4.66	447
10	15	193	39	129	1.43	4.65	326
			standard error		.10		17
		difference required to be significant			.31		58
Experiment 2 — 84 to 127 lbs.							
13	15	136	35	82	1.34	3.53	269
13	20	121	31	85	1.39	3.42	252
13	25	125	33	86	1.28	3.58	284
			standard error		.05		15
		difference required to be significant			.16		45
		84 to 156 lbs.					
13	15	136	53	82	1.39	3.88	281
13	20	121	50	85	1.47	3.83	265
13	25	125	54	86	1.31	3.89	300
			standard error		.05		16
		difference required to be significant			.13		48
		127 to 200 lbs.					
7	15	166	39	128	1.76	4.71	268
7	20	154	46	128	1.73	4.58	265
7	25	162	45	127	1.49	4.60	316
			standard error		.06		19
		difference required to be significant			.19		59
		84 to 200 lbs.					
13	15	136	77	82	1.55	4.28	276
13	20	121	77	85	1.55	4.28	276
13	25	125	77	86	1.38	4.29	314
			standard error		.04		12
		difference required to be significant			.12		33
Experiment 3 — 40 to 74 lbs.							
9	12	77	76	40	.45	1.56	375
12	17	70	72	38	.50	1.43	301
12	22	73	70	41	.51	1.31	289
			standard error		.03		29
		difference required to be significant			.10		85
		74 to 156 lbs.					
7	12	156	73	73	1.14	3.25	285
12	17	144	58	75	1.43	3.28	229
11	22	145	69	75	1.18	3.29	274
			standard error		.04		11
		difference required to be significant			.15		33

TABLE 2—*continued*

NUMBER OF PIGS	AMOUNT OF PROTEIN IN RATION	AVERAGE AGE AT BEGINNING	LENGTH OF EXPERIMENTAL PERIOD	INITIAL BODY WEIGHT	MEAN AVERAGE DAILY GAIN	MEAN AVERAGE DAILY RATION	FEED FOR EACH 100 LBS. GAIN
	%	*days*	*days*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
		156 to 213 lbs.					
4	12	213	35	155	1.68	4.64	283
11	17	202	33	156	1.83	4.61	257
6	22	214	38	155	1.52	4.66	309
				standard error	.06		13
	difference required to be significant				.17		38
		130 to 210 lbs.					
5	12	209	53	130	1.59	4.63	282
12	17	187	49	131	1.84	4.52	245
10	22	193	55	128	1.56	4.47	302
				standard error	.07		11
	difference required to be significant				.20		32
		40 to 210 lbs.					
9	12	77	166	40	.88	2.79	332
12	17	70	166	38	1.09	2.76	252
11	22	67	166	41	.98	2.75	282
				standard error	.04		16
	difference required to be significant				.13		48
Experiment 4 — 33 to 70 lbs.							
10	17	78	54	33	.75	1.85	271
10	22	78	51	32	.74	1.94	270
10	27	75	49	34	.78	1.98	254
				standard error	.12		10
	difference required to be significant				.37		31

From the results obtained with pigs of lighter weight, 80 to 125 pounds, 40 to 74 pounds and 33 to 70 pounds, no single level of protein intake could be designated as being the most satisfactory for body gain but the 12% level was definitely unsatisfactory with respect to feed requirement. Woodman and associates ('36) found that "extra protein" was of no value in improving the rate of growth in the period from weaning to 90 pounds, a period in which the value of such protein might be expected to be manifested. It is possible that other factors, some of which may be unrecognized, may influence the growth of pigs at this stage of their growth, more than it is influenced by the protein level, providing that the protein is adequate from the viewpoint of quality. The results of these experiments do not indicate any deficiency in the

quality of the protein or any of the rations with the possible exception of the 10% protein ration used in experiment 1.

The results obtained with the lighter pigs are not surprising in view of the difficulties often encountered with pigs of this weight. The general nutritional requirements of such pigs are not very well established and for this reason many workers select pigs for growth studies when they weigh 70 to 80 pounds rather than at weaning time. In the interim between weaning and being placed on experiment, these larger pigs will have had access to feeds which do not impose on them the restrictions of an experimental diet and thus may be able to build up reserves of dietary essentials which may have been depleted during the suckling period. The less thrifty individuals are eliminated during this period and the surviving pigs which attain a weight of 70 to 80 pounds and which are then placed on the experimental regime, can usually be depended on to give a more satisfactory growth response. The findings of Chick and associates ('38 a, b, c) and Hughes ('38, '39) with respect to the requirements of pigs for members of the vitamin B complex illustrate the paucity of knowledge relative to the requirements of swine for specific dietary factors.

Woodman and associates ('36) have conducted individual feeding tests in a manner similar to that of these experiments. In addition these investigators conducted group feeding tests with the same feeds. From their data they have concluded that the group feeding technique, while it lends confirmation to the individual tests, may be of very limited value, due to its lack of sensitiveness, especially when the expected differences to be investigated are small.

It may be argued that differences too small to be detected by the group feeding method may be too small to have any practical significance; nevertheless, the individual feeding method has certain outstanding advantages. The quantity of feed consumed by each pig is known as is its effect on the individual, and at least an attempt can be made to correlate differences in individual performance with food consumption. Such differences may be brought out by the group feeding

method, but they may be so obscured by the variability of the individual response among the various group members, that definite assignment of them to feed is impossible. Thus the group feeding method is to be regarded as a link between practical feeding and scientific experimentation and as a means of verification under practical conditions of the individual feeding trial.

The present experiments yield some interesting data on the quantities of feed required per 100 pounds of body increase. In these experiments the average figure for this value is of the order of 285 pounds, the range being from 447 pounds on the 10% protein level (experiment 1) to 229 pounds on the 17% protein level (experiment 3) at body weights from 70 to 156 pounds. These figures give some idea of the efficiency with which feed is converted into body tissue and, on the whole, are much lower than those usually encountered in either group feeding work or under practical feeding conditions. While no definite average figure is available for actual practical conditions with efficient feeding, approximately 400 pounds of concentrates are usually required to produce 100 pounds of body gain. Less than this quantity of feed may be required in many cases but feed requirements below 300 pounds are unusual in general feeding practice.

The system of feeding used in these experiments has consistently given results for feed efficiency of the order herein reported and at the same time the rate of growth has been maintained satisfactorily. It is recognized that in the effort to maintain an equalized feed intake between three pigs, the feed intake of two members of the triplet was limited by that of the third pig, and that in a sense, limited feeding was being practiced on these two pigs. Under such conditions it is apparent that the efficiency of food conversion might have been almost at a maximum, there being no waste and no feed consumed merely in satisfaction of appetite.

Woodman and associates ('36) observed greater thriftiness among pigs fed individually as compared to those fed in groups, the former making the greater gains in the least number of

days. These authors suggest that there is a considerable saving of energy when pigs are individually fed as compared to group-fed pigs which by nature use up considerable energy in consequence of "scrambling" at feeding time at the common trough. The logic of their deductions is recognized and this point together with the possibility of a limited feed intake may explain the high efficiency of food conversion obtained in these experiments.

It should be further pointed out, however, that the average daily gains in experiment 2 exceeded those of experiment 3 in practically all instances, a result which may be due to starting the pigs on experiment 3 at a lighter weight (40 pounds) rather than at 80 pounds as in experiment 2. The resulting initial growth of the pigs on experiment 2 was slow due possibly either to an unrecognized deficiency of the ration or to limited initial reserves of some of the pigs.

SUMMARY

The optimum quantity of protein in the ration for pigs for the growth span from 40 to 210 pounds was found to be from 15 to 17%. Levels of protein of 20, 22, 25 and 27% were not outstanding in their superiority to the 15 and 17% levels, but the trend of the results favored the higher levels of protein intake for pigs from weaning up to a weight of approximately 75 pounds. The lower levels of protein intake were superior to the higher levels for pigs between the weights of 120 and 200 pounds. Rations containing 10 and 12% of protein were unsatisfactory.

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